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THE ORAL ADMINISTRATION OF HORMONAL PROTEINS

THYROID PROTEIN AND INSULIN[1]

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A great impediment to endocrine therapy is the futility of oral medication. This is largely due to the protein nature of many hormones. To be sure, some clinicians have felt that huge doses might be effective. Most controlled observations in animals, however, have not shown this to be the case.

It has usually been said that such protein molecules were inactive because they were disrupted by the enzymes of the digestive tract. Nevertheless, studies on thyroid protein and on insulin,[3] conducted in this laboratory, suggest certain qualifications of this statement. First, enzymic inactivation of these proteins may be reversible. Secondly, as is already known for thyroid protein, both of these hormones can produce consistent effects by mouth. There is even evidence that their split products may be active.

THEORETICAL

The oral administration of thyroid protein. Previous studies of thyroid protein offer interesting suggestions for studying other hormonal proteins. Thyroglobulin is hydrolyzed by proteolytic enzymes, but its fragments can be recombined successfully. Thus, Lerman and Salter (1) showed that enzymic disruption of the thyroid protein molecule in vitro destroyed much of its activity. In fact, about two-thirds of the material was degraded to a relatively inactive peptone of diiodotyrosine. In other words, a molecular weight of 700,000 (2) was reduced to about 5,000 by fragmentation. The small pieces, however, could be built back to a larger molecule by enzymic synthesis. The artificial protein so formed had an average molecular weight of only 18,000, but it proved to be higly potent. These experimental effects suggest, of course, that enzymic synthesis may work similarly in the organism. Enzymes presumably are essential to the elaboration of thyroid protein in the thyroid

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[2] Aided by a grant from the Ella Sachs Plotz Foundation to William T. Salter.
[3] Insulin used in these experiments was kindly supplied by Eli Lilly and Company.

gland: may they not also aid in the successful assimilation through the intestinal wall?

The oral administration of insulin. It is the purpose of this paper to show that insulin behaves like thyroid protein in the features just mentioned. The similarity is qualitative, not quantitative. Insulin is generally said to be 'inactive when taken by mouth.' Nevertheless, a number of investigators have obtained positive results. In no case, however, have hypoglycemic reactions been obtained in intact animals, consistently and reproducibly, by methods consistent with normal gastro-intestinal physiology.

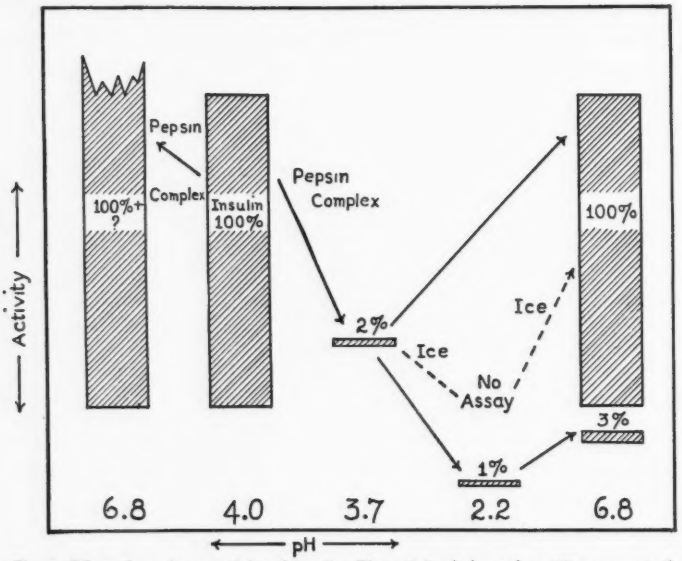

Fig. 1. Effect of pepsin on activity of insulin. The activity designated as 100 per cent is the response to 0.0012 mg. crude insulin (0.013 I.U.).

These results were reviewed in 1933 by Eaton and Murlin (3). Murlin and co-workers have also definitely shown absorption of insulin in depancreatized dogs when the hormone was administered by stomach tube. The hormone was 'protected' by certain chemicals, e.g., calcium lactate and sodium bicarbonate (3) or blood serum (4).

The effect of proteolytic enzymes on activity of insulin. If insulin is to exert its action after intestinal absorption, it must run the gauntlet of proteolytic enzymes and escape total destruction. Now, enzymic inactivations of insulin in vitro have hitherto been reported as rapid, and essentially irreversible. Pepsin, trypsin, and papain will all cause marked inactivation before hydrolysis is complete (5, 6). Attempts to resynthesize such fragments into an active 'plastein' by peptic synthesis have failed (7). It has been claimed (8) that some regeneration of activity can be accomplished by alkalinization after peptic action, but any appreciable reactivation has been denied (5). In the present report, however, evidence is presented that the

TABLE 1. SUBCUTANEOUS ASSAYS OF PEPSIN-INSULIN COMPLEX. INACTIVATION AND REACTIVATION

pH		Standard Insulin Doses Given	No. of Mice	% Convulsions	% Activity
Initial	Assayed				
6.8	6.8	1	25	70	100+
6.8	6.8	½	30	25	
2.2	2.2	100	25	20	1
2.2	6.8	30	25	50	3
3.7	3.7	50	25	50	2
3.7	6.8	1	30	50	100
2.2 (at 0°C.)	6.8	1	24	45	100

first stage of enzymic action may be entirely reversible. This is true even though the hormone has been almost completely inactivated.

The details are given in protocol 1. They indicate that at pH 4.0 pepsin combined with insulin to form a precipitate. This precipitate preserved the full activity of its insulin moiety when injected subcutaneously. At pH 3.7, however, the activity was reduced to 2 per cent of the original value. Nevertheless, the original potency could be restored within 5 minutes at pH 6.8. When the mixture was acidified to pH 2.2 at room temperature, irreversible loss of activity rapidly ensued. This effect of pepsin in strongly acid solution could be prevented by cooling to 0°C. These changes are summarized in figure 1 and table 1. The test animals were pedigreed mice.

Insulin formed similar complexes with other proteins, i.e., serum proteins and egg albumin, and with beef peptone. These precipitates differed from that formed with pepsin, however, in that changes in acidity failed to alter the activity.

Even after rather drastic hydrolysis by pepsin, the 'destroyed' insulin could be recovered in part by the addition of alkali. The details are given in protocol 2. The peptic split-product showed a distinct isoelectric point near that of insulin, but was far less soluble at pH values below 5. When assayed by subcutaneous injection, the split-product showed only 3 to 10 per cent the activity of the original insulin. After reactivation with alkali, the activity increased to 10 to 28 per cent of the original. The assays are summarized in table 2.

TABLE 2. SUBCUTANEOUS ASSAYS OF A PEPTIC HYDROLYSIS PRODUCT[1]

Initial				Reactivated		
Experiment No.	% Convulsing	Activity % of Ins.	Fraction of Initial Dose	% Convulsing	Activity %	Reactivation
1	90	3	0.17	10	10	3.—fold
2	90	2	0.17	90	13	6.5
3	60	5	0.40	60	12	2.5
4	30	2	0.20	50	12	6
5	60	5	0.33	20	12	2.5
6	60	10	0.50	40	16	1.5
7	40	14	0.50	40	28	2
8	40	3	0.50	40	6	2

[1] The activity of the initial precipitate formed during heptic hydrolysis is compared with that of the same preparation after reactivation.

The effect of insulin by mouth. The foregoing experiments suggested that oral administration of insulin should be feasible. To be sure, preliminary experiments were discouraging. When huge doses of insulin were dissolved in distilled water and given to mice by stomach tube, no effect was ordinarily observed. Even repeated half-hourly doses 3200 times the 'standard skin dose' were ineffective. Both the usual pharmaceutical insulin, 80 U, and protamine-insulin (Squibb, dispensed for clinical trial)[4] were ineffective orally.

When, however, insulin hydrochloride as defined by du Vigneaud (9, p. 531) was given in aqueous solution, a constant and reproducible effect was obtained.[5] Figure 2 shows that, in one experiment comprising 55 consecutive animals, 53 showed typical hypoglycemic convulsions. Blood-sugar determinations in the 50 convulsing mice averaged 34 mg. per cent. Of the two non-convulsing animals, one had a blood sugar of 41 mg. per cent. The animals become normal promptly after glucose solution was administered subcutaneously. To obtain convulsions in 50 per cent of such animals, a standard dose of 1.8 mg. of crude insulin (20 I.U.) was required.

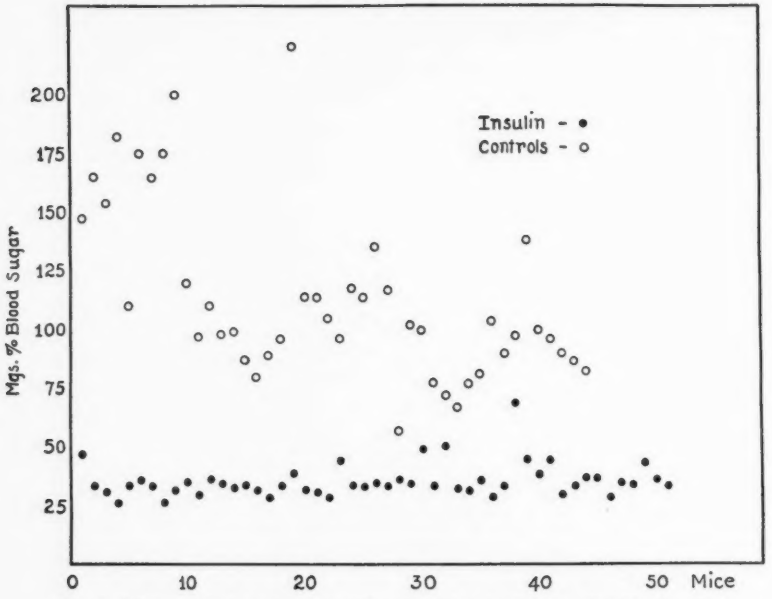

Fig. 2. Effect on blood sugar of consecutive mice when insulin was administered orally. The dose was 5.7 mg. crude insulin (62 I.U.)

The precise rôle of the acid cannot be stated definitely at present. It probably did not permanently alter the protein molecule, because after neutraliza-

[4] The protamine preparation was kindly donated from that allocated to Dr. Alexander Marble.

[5] This may be prepared in one of two ways. The precipitate obtained in 1 N hydrochloric acid may be centrifuged off and dissolved in water, thus yielding a free HCl concentration of about 0.05 N; or acid insulin may be dissolved in the same concentration of HCl.

tion the insulin behaved normally. Moreover, the acid did not appear to acidify the intestinal contents of the animals sufficiently to inactivate trypsin. At autopsy 20 minutes to 3 hours after the usual dose of acid, the duodenal contents of these animals were no more acidic than pH 6.0 to 6.4. Furthermore, deliberate alkalinization did not enhance the oral effect. Indeed, insulin given by stomach tube in 1 per cent sodium bicarbonate and 2 per cent calcium lactate (3) was ineffective in a dose of 1.5 mg. (17 I.U.), i.e., three times the standard dose for 50 per cent convulsions in this strain.

The Effect of Ferric Salts on the Activity of Insulin by Mouth

Effect of insulin hydrochloride and iron. Insulin hydrochloride was prepared by mixing insulin with 1.5 per cent hydrated ferric chloride (hexahy-

TABLE 3. INSULIN BY MOUTH—INDIVIDUAL ASSAYS

Insulin Hydrochloride[1]			Insulin and Ferric Chloride			Insulin and Ferric Am Citrate		
Black Mice—C-57 strain			Black Mice—C-57 strain			Swiss Mice		
Dose mg.	No. of Mice	% Convulsions	Dose mg.	No. of Mice	% Convulsions	Dose mg.	No. of Mice	% Convulsions
1.0	5	0	0.25	5	0	0.12	10	20
1.0	5	0	0.5	8	60	0.18	10	20
1.5	5	20	1.0	10	80	0.18	5	40
1.5	6	20	2.0	4	100	0.24	6	50
1.6	4	50				0.24	10	90
1.5	5	80	Swiss Mice[2]			0.50	6	83
2.0	5	40				0.80	7	87
2.0	5	60	0.05	5	0			
2.0	5	60	0.06	5	0			
2.0	5	60	0.06	5	20			
2.0	5	60	0.08	5	40			
			0.10	5	40			
			0.10	5	40			
			0.12	5	40			
			0.12	5	60			
			0.16	5	60			
			0.20	5	40			
			0.35	5	40			

[1] Selected from a total of 18 assays.
[2] Selected from a total of 39 assays.

drate). For this mixture the oral dose requisite for 50 per cent convulsions was 0.5 mg. of crude insulin (5.5 I.U.), or about one-fourth the value for the the hydrochloride alone.

In all the assays quoted thus far, black mice of the C-57 strain were used. When inbred albino mice of the 'Swiss' strain were tested, they were found to be more responsive to oral insulin with ferric chloride. In these animals the average dose for 50 per cent convulsions was 0.10 mg. (1.1 I.U.). By subcutaneous assay, they showed substantially the same sensitivity as the black, C-57 strain. The assays are summarized in table 3.

Neutral insulin and iron. Acid is not essential to an oral effect. This was shown in experiments with a solution of insulin in 2.5 per cent ferric ammonium citrate at pH 6.[6] In Swiss mice, a dose of 0.2 mg. (2.2 I.U.) was

[6] Ferric chloride was not used because ferric hydroxide is precipitated near neutrality. A similar technic was used by Maxwell and Bischoff (12) when administering insulin subcutaneously.

required to produce convulsions in half the animals. This dose is only slightly larger than that with the acid iron salt, as indicated in table 3.

Relative efficiency of oral administration. These results showed that insulin can produce marked physiological effects when given by mouth under suitable conditions. More important is the constancy of the activity.

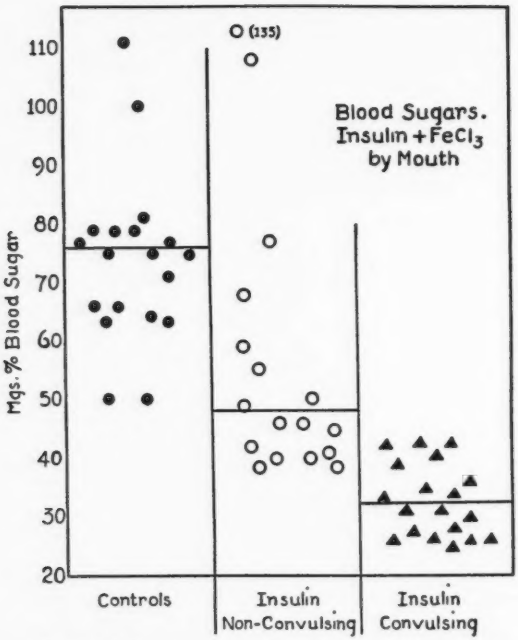

Fig. 3. Blood sugars of animals which did and did not show convulsions after oral insulin. The average dose was 0.22 mg. crude insulin (2.4 I.U.) for the mongrel animals used in this particular experiment.

TABLE 4. 'STANDARD' INSULIN SKIN AND MOUTH DOSES

Strain of Mice	Preparation Given	Skin Dose mg.	Mouth Dose mg.	Mouth/Skin Ratio
Black—C-57	Insulin	0.0012		
Black—C-57	Insulin HCl		1.8	1500
Black—C-57	Insulin—$FeCl_3$		0.5	400
Albino Swiss	Insulin	0.0011[1]		
Albino Swiss	Insulin—$FeCl_3$		0.10[1]	90
Albino Swiss	Insulin—FeAmCit.		0.20	180

[1] This dose produced convulsions in 40% of animals.

In table 4 the efficiency of oral insulin is compared with that of subcutaneous insulin. In interpreting the data, certain important limitations in their significance must be borne in mind. The 'mouth : skin ratios' show that much larger doses are needed for oral effects. As explained below, how-

ever, these ratios should not be applied directly to clinical diabetes. In short, these values apply only to a highly artificial, laboratory status in normal mice of specified strains.

The dynamics of oral insulin effects. The reason for this statement becomes clearer from a consideration of the dynamics of the insulin effect, shown in figure 3. The median value for blood sugar in fasted control mice was 76 mg. per cent. The oral dose was so adjusted that only 50 per cent of the test animals convulsed. The median blood-sugar value for convulsing animals was 32 mg. per cent. One-half hour after the last convulsion, blood was taken from all the non-convulsing animals. Their blood

TABLE 5. PAPAIN DIGEST OF CRUDE INSULIN: EFFECT BY MOUTH AND SUBCUTANEOUSLY

Experiment No.	Mg. Skin dose[1]	Mg. Mouth Dose[1]	Ratio of Dose Mouth/Skin	Insulin Ratio[2] / Digest Ratio
1	0.0036	0.18[3]	50	1.8
2	0.0084[3]	0.20[3]	24	3.8
3	0.0056 plus	0.18	32 max.	2.8 min.
4	0.0056	0.24	43	2.1
5	0.0108[3]	0.36[3]	33	2.7
6	0.0108	0.44	40	2.3
7	0.0120	0.44	37	2.4
8	0.0100	0.56[3]	56	1.6
9	0.0096	0.56	58	1.5
10	0.0072[3]	0.40	55	1.6
11	0.0054	0.40	73	1.2
12	0.0072[3]	0.56	77	1.2
Average	0.008 or 0.09 I.U.	0.38 or 4.2 I.U.	45	2.0

[1] This dose produced convulsions in 40% of fasting animals.
[2] Insulin itself is 90 times as effective subcutaneously as orally; the digested insulin about 45 times. Hence, digestion has decreased the ratio two-fold.
[3] Estimated by procedure described in protocol 3.

sugar was definitely lower than the control value: their median value was 48 mg. per cent.

In short, all the mice in the test group had probably been absorbing active insulin, but some at a less effective rate than others. The dose required to produce 50 per cent convulsions, therefore, is a highly arbitrary criterion for determining the efficiency of oral administration. It does not apply quantitatively to clinical or experimental diabetes.

The effect of enzymic hydrolysis on oral insulin activity. The proteolytic enzyme, papain, was shown by Freudenberg (6) to produce only 70 per cent inactivation of insulin even after prolonged action. Such preparations were made as described in protocol 3. Twelve separate experiments with these papain digests showed that digests of insulin could retain activity. They also suggested that the small fragments of hydrolyzed insulin might be absorbed through the intestinal wall more effectively than the whole insulin molecule.

As shown in table 5, simultaneous subcutaneous and oral assays were made, controlled by appropriate assays of whole insulin in the same group of inbred animals. In every case the 'standard' oral dose for the papain

digest was higher than that for unchanged insulin. Nevertheless, the digest was more effective than whole insulin in terms of its respective subcutaneous dose. Thus, for whole insulin in ferric chloride, the oral: subcutaneous ratio was 90. For digested insulin, by contrast, the oral : subcutaneous ratio was 45. Indeed, this last value ranged in various experiments from 24 to 77.

EXPERIMENTAL

Experimental procedures. The insulin used in these experiments was an amorphous powder with an activity of 21-22 American units (11 I.U.) per milligram.[7] All assays, unless otherwise stated, were conducted with male mice from two inbred strains. They were fasted 16 to 24 hours before use. At least a week was allowed to elapse between assays with the same animals. After injection or intubation, the mice were placed in a well-ventilated incubator at 37°C., and the response was followed for two hours according to the method of Trevan (10).

Blood sugars were estimated by a modification of the micro-method of Folin (11). For this purpose one-tenth cc. of blood was drawn from the tail. No anesthetic was needed with convulsing or comatose mice. All subcutaneous injections were given in the back, in a volume of 0.5 cc. of isotonic saline. Intubations into the stomach were done by means of a suitably shaped glass tube affixed to the end of a syringe. The desired dose was dissolved in 0.4 cc. of solution. If regurgitation occurred, or if there was any possibility of having scratched the esophagus, or entered the bronchial tree, the mouse was discarded. In skilful hands, these accidents were rare.

Control mice were fasted, given 0.4 cc. 0.1 N HCl by stomach tube, and placed in the incubator for the same period as those given insulin by mouth, i.e., 30 to 50 minutes. Most of these animals were anesthetized by a mild dose of chloroform before drawing blood. Nevertheless, the high blood sugars obtained in these animals suggested that the excitement preliminary to anesthesia, as well as the chloroform itself, might be inducing hyperglycemia. Accordingly, a second group of control animals was anesthetized with nembutal before being intubed, and blood was drawn while they were still flaccid in anesthesia. The results were generally slightly lower by this method.

Standardization of insulin activity. The 'standard skin dose' of the various insulin preparations was defined as that dose which would cause 5 out of 10 mice to convulse within 2 hours. For the original insulin powder, many such assays were conducted, and the dose plotted against percentage of convulsions obtained. The resultant curve yielded the 'standard' subcutaneous doses of table 4. These were confirmed from time to time.

In the case of the peptic hydrolysis product, in which a precipitate was separated off, it became impossible to estimate the insulin dose on a direct basis. Therefore, total nitrogen determinations were used as a parameter. The 'standard' nitrogen dose of our original insulin was 0.00015 mg. To determine the activity of the various preparations made from insulin, the dose causing

[7] Kindly supplied by Eli Lilly and Company.

an incidence of 50 per cent convulsions was determined. This dose was then compared with that of the untreated insulin.

Determinations of pH. All pH values quoted were found by testing a drop of the material in question on a porcelain plate with suitable indicators, and comparing the color obtained with buffer mixtures of known pH.

Protocol 1

A pepsin-insulin complex. Equal volumes of insulin solution in water, containing 28 mg./cc., and pepsin solution containing 200 mg./cc., were adjusted to pH 4 and mixed. A copious precipitate was obtained. The ratio of insulin to pepsin (Merck) was roughly 1:7. Smaller amounts of pepsin did not always give maximum precipitation of the insulin.

The precipitate resembled insulin in its solubility above pH 6, but was less soluble on the acid side of the iso-electric point. It required a pH of 3 for complete solution.

When the precipitate was centrifuged and washed free of excess pepsin, it failed to digest itself even after several hours at 37°. Excess pepsin slowly dissolved it, however, even at pH 4.0 to 5.0. The nature of this latter process is obscure, because pepsin is usually considered to be inactive hydrolytically at pH 4.

For subcutaneous assays, the complex mixture was adjusted to the desired pH, and a suitable aliquot added to isotonic saline solution adjusted to the same pH.

Protocol 2

A peptic hydrolysis product. Six mg. of crude insulin powder were dissolved in 0.28 cc. of 0.3 N HCl, and 0.28 cc. of a 0.3 per cent solution of pepsin in 0.3 N HCl added. This corresponds to 0.84 mg. pepsin, or about one-seventh the weight of crude insulin.

The mixture was maintained at 37° for one-half hour. Usually a precipitate appeared after about 15 minutes. On centrifuging, it was found that the supernatant fluid was not always clear, but continued to form more precipitate. This difficulty could be obviated by shaking during incubation.

After centrifuging four minutes at 2200 r.p.m., the supernatant fluid was decanted off, the precipitate washed with 0.5 cc. 0.3 N HCl, and centrifuged again. A second decantation followed.

Inasmuch as the pepsin-nitrogen was constantly less than one-seventh of the total nitrogen, total nitrogen determinations on an aliquot of the precipitate suspension showed that a remarkably constant fraction of the original insulin was obtained. Fifty to 55 per cent of the nitrogen was almost always found, and lower yields could usually be correlated with incomplete precipitations. Increasing the time of incubation up to 20 hours failed to increase the yield.

Preparation of the initial material for assay. One cc. of distilled water was added to the washed precipitate, whereupon it dissolved somewhat, and dilute NaOH was then added until the suspension reached a pH of 4.5. Complete solution of the precipitate did not occur at this acidity in the 4 to 5 cc. volume used, whereas insulin would have been entirely in solution. With the least possible delay, an aliquot was removed from the suspension at pH 4.5 for assay.

Reactivation. To the remainder of the suspension, alkali was now cautiously added. At pH 5-6 a distinct isoelectric precipitation was observed, and at about pH 6.5 all the precipitate dissolved. The solution was brought to pH 8 for nearly one minute, and then adjusted to pH 6.0, where a slight re-precipitation occurred. This material was maintained at 5 to 8°C. for 17 hours before assay.

The suspended material present in both initial and reactivated preparations dissolved on dilution with isotonic saline before injection.

Protocol 3

Preparation of a papain digest. Six mg. of crude insulin were dissolved in 1 cc. of water. Eighty mg. Merck's Papain was thoroughly mixed with 3 cc. of water, and the mixture centrifuged. The supernatant fluid containing active enzyme was then decanted free from insoluble matter. This procedure was next repeated with 1 cc. of water. The combined enzyme solutions were added to the insulin solution with stirring, and the total volume adjusted to exactly 5 cc. A precipitate appeared. The mixture was immediately transferred to a small Erlenmeyer flask, and placed in a water bath at 37.5°C., where it was agitated by a mechanical rocker. After exactly 20 minutes, when the digest was practically clear of precipitate, it was removed, and

rapidly cooled under a tap. Aliquots were immediately removed for assay. If assays requiring more than 6 mg. insulin were planned, this procedure was carried out in duplicate and the digests combined at the end of incubation.

Assays. Aliquots for oral assay were adjusted to pH 2, and diluted with water so that the dose was contained in 0.4 cc. volume. Then $FeCl_3$ was added to 1.6 per cent concentration.

All doses were prepared and administered as quickly as possible so as to minimize further hydrolysis. Groups of 5 fasted Swiss mice were used for each individual dose. For the digest, 3 doses were tested simultaneously both orally and subcutaneously. For the insulin controls, 2 doses were similarly tested on the same day with fasted mice from the same lot. Thus 50 mice were used to obtain a single result.

Since a 50 per cent response cannot be obtained with a 5-mouse assay, we adopted 40 per cent as our standard response in these experiments. Oral doses were planned with the expectation that the lowest dose would be entirely negative, because the response to oral insulin differs from that administered subcutaneously in not showing a regular increase with increase in dosage. After oral administration, the response was not predictable beyond a certain threshold dose. This threshold, however, was rather sharp and uniform, and consisted in a sharp jump from 0 to 40-50 per cent convulsions when the dose was slightly increased. It served, therefore, as a convenient end point in our biological assays. A somewhat similar procedure was used for papain digests assayed subcutaneously. In this case, also, doses over 10 times the insulin standard produced no regular increase in the percentage of convulsions. Indeed, 60 per cent convulsions was the highest value ever observed with any dose.

If a given activity was such that our assays did not yield exactly a 40 per cent response, we plotted the actual points obtained. Then we either interpolated to 40 per cent the lines connecting these points, or drew a line to 40 per cent having the average slope of all such determinations.

DISCUSSION

The dynamics of insulin utilization after oral administration deserve special comment. The process differs appreciably from that after subcutaneous injection. These two different mechanisms were revealed by differences in the respective convulsive responses occurring within two hours after administration. First, the latent period following a standard oral dose was often less than half an hour; whereas the latent period after subcutaneous injection was twice that interval. Second, when given orally with iron, the effective oral dose of insulin continued to act for a very considerable time. Even when half the mice showed no ill effects whatever, those which convulsed would often relapse into convulsions a second or even third time despite glucose therapy. Such relapses were never observed after a subcutaneous dose.

The explanation of these differences can be given in terms of the rates of several interrelated processes. The result of any dose of insulin probably depends upon a balance between these several rates. In particular, the rate of absorption into the blood is balanced against the rates of destruction and excretion. With oral administration the rates of absorption from, and destruction in, the digestive tract become of dominating importance. Consequently, a large amount must be given in order to obtain an effect. To be sure, this hypoglycemic effect probably is due to only a small fraction of the huge dose, which serves as a reservoir of hormone. Hence, small variations in the rates of these processes will produce large variations in the final insulin effect.

A comparison of the blood-sugar values listed below will clarify these points.

Normal fasting blood sugar ..... 76 mg. %
Blood sugars of 6 mice during a second convulsion after glucose, at from 2¼ to 4 hours after oral insulin ..... 40 mg. % avg.
Blood sugars of 7 mice which convulsed once and received glucose, at from 2½ to 4 hours after oral insulin ..... 58 mg. % avg.
Blood sugars of 6 control mice which received glucose at the same time as the above mice .....169 mg. % avg.

It is evident that insulin might be absorbed so fast that the blood-sugar level remained extremely low for some hours. Under these circumstances, injected glucose gave only temporary relief. In fact, some hypoglycemic animals failed to respond to glucose at all.

It is likewise probable that orally administered insulin is not necessarily altogether destroyed by intestinal enzymes within two hours after administration.

SUMMARY

The 'inactivation' of insulin by proteolytic enzymes in vitro may be reversed in whole or in part. This finding suggests that the oral administration of insulin may be effective under appropriate circumstances.

In fact, the oral administration of insulin preparations in mice has consistently and reproducibly produced hypoglycemic coma or convulsions within two hours after administration. The oral dose observed for undigested insulin hydrochloride was about 90 times its subcutaneous dose. The oral dose observed for digested insulin was about 45 times its subcutaneous dose. Iron salts increased the effectiveness of insulin and digests of insulin when administered orally.

These observations suggest qualitative similarities between thyroglobulin and insulin with respect to the action of proteolytic enzymes. They suggest, further, that hormonal proteins can be used orally under suitable conditions.

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EFFECT OF HYPERTHYROIDISM UPON GROWTH AND CHEMICAL COMPOSITION OF BONE[1]

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By use of exact balance studies Aub, Bauer, Heath and Ropes (1) demonstrated that calcium and phosphorus excretion are markedly increased in patients with hyperthyroidism, and that the same phenomena could be induced by feeding thyroid to normal individuals. This excessive excretion of calcium in hyperthyroid states has been reported in other clinical and experimental studies (2, 3, 4, 5, 6). In order to account for these findings and for the osteoporosis observed in hyperthyroidism by themselves and others (4, 7, 8, 9, 10), Aub, et al. (1, 11) postulated that thyroid has a specific stimulating catabolic effect on the calcium deposits in the bones. They pointed out that most of the other necessary body elements are not readily excreted to the degree of producing comparable negative balances. Parhon, Derevici and Derevici (12) reported slightly lower calcium content in the bones of the treated animals after feeding thyroid to 12 dogs over a period of 3 weeks. The considerable variation among the controls renders their results of doubtful significance. Hoskins (13) noted that the bones from rats fed thyroid appeared slightly heavier than those of the controls in the wet and dry state. Thompson (14) reported a decrease in bone ash from rats in which hyperthyroidism was induced by high calcium-low iodine diets.

The studies here reported were undertaken in an effort to determine, by chemical and histological observation, whether a specific effect of thyroid upon the calcification of the bones of rats could be demonstrated. The study led to the observation of the effect of thyroid upon growth at the epiphyseal junction of the long bones, which is also reported.

Bircher (15) fed young rats with thyroid for 4 months and, by means of x-ray and histological examination, demonstrated a retardation of growth with almost complete closure of the epiphyses of the long bones. Dott (16) noted by x-ray study a 28 per cent increase in the rate of growth of femurs from 2 puppies fed thyroid. The final bone length and stature were fixed at a subnormal limit because of premature epiphyseal ossification, observed histologically. Gudernatsch (17) fed thyroid to tadpoles and found that the treated animals underwent metamorphoses earlier than the controls, but always remained much smaller in size. Hoskins (18) found that injection of the monoacetyl derivative of thyroxin into rats inhibited growth in length and weight, but reported that x-rays showed an acceleration of 'epiphyseal activity,' without closure of the epiphyses.

[1] This work was aided by a grant from the Josiah Macy, Jr. Foundation.

With the exception of the work of Aub and his collaborators and that of Cope and Donaldson, clinical observations on the effect of thyroid on calcium metabolism and on the growth of bone are, in general, poorly controlled and inconclusive. In the reports of hyperthyroidism in children, only Bloom (19), with 26 cases under 14 years of age, noted growth as distinct from body weight, and stated that there was a 'general retardation.' Experimental work is likewise incomplete. Clinical use of thyroid to promote increase in stature there-

TABLE 1. COMPARISON OF DATA OBTAINED BY ANALYSIS OF FEMURS FROM UNTREATED RATS WITH THOSE FROM RATS TREATED WITH THYROID

	Rats killed at 4½–5 months of age				Rats killed at 7–8 months of age			
	Means and Standard Deviations		Differences	P	Means and Standard Deviations		Differences	P
	6 Untreated	11 Treated			10 Untreated	12 Treated		
Age at death, months	4.8	4.7			7.7	7.8		
Weight at death, gm.	185.0± 10.4	172.0± 21.6	−13.0	9.5	304.0± 19.9	217.0± 41.5	−87.0	0
Observed weights, gm.								
Whole femur	.5009± .044	.5276± .044	.0267	25.0	.6966± .070	.7531± .058	.0565	5.2
Water	.1935± .016	.2146± .021	.0211	2.4	.2573± .027	.2916± .013	.0343	0
Fat	.0108± .002	.0097± .003	−.0011	42.4	.0134± .003	.0120± .002	−.0014	23.0
Ash	.1886± .022	.1907± .019	.0021	85.7	.2729± .038	.2853± .035	.0124	45.2
Residue	.1080± .011	.1126± .010	.0046	41.8	.1530± .012	.1642± .015	.0112	6.4
Calculated ratios, percentage								
Water/whole femur	38.8 ±2.16	40.6 ±2.53	1.8	14.0	37.0 ±1.78	38.8 ±1.56	1.8	1.6
Ash/dry, fat free femur	63.5 ±1.01	62.8 ±1.34	−0.7	24.0	63.9 ±2.42	63.3 ±2.22	−0.6	56.8
Ash/residue	1.74± 0.08	1.69± 0.09	−0.05	20.1	1.78± 0.19	1.74± 0.11	−0.04	54.6

fore rests upon an insecure foundation, especially in view of the reported inhibitory effect of this substance upon the growth of long bones.

EXPERIMENTAL

The experiments consisted in feeding thyroid to, *a)*, young rats during the period of active growth and *b)*, older rats after decrease in active growth. Bills's stock diet (20) was used throughout. Weights of the animals were recorded for 3 weeks previous to adding thyroid to the diet, and then Armour's desiccated thyroid of 0.3 per cent organic iodine content was mixed with the dry food given to the experimental rats. In each case the purpose was to produce the maximum hyperthyroidism compatible with the life of the animal. Criteria of the extent of hyperthyroidism consisted in mortality rate, which averaged between 20 and 30 per cent, weight loss in the older animals and retardation of weight increase in the younger ones, diuresis, polydipsia, polyuria, tachycardia and irritability. Changes of as little as 0.1 per cent in the thyroid content of the diet usually produced definite weight and mortality changes, indicating that the method of administration was roughly quantitative and uniformly effective. The rats were fed diets of thyroid content ranging between 0.4 and 0.75 per cent for varying periods between 4 to 12 weeks. The thyroid treatment was started between the ages of 1.5 and 5.5 months and the animals were killed between 4.5 and 8 months of age. A diet with over 0.7 per cent thyroid content proved too toxic to support the life of the rats after 5 weeks of its administration.

Individual experiments were carried out on litter mates or on combined

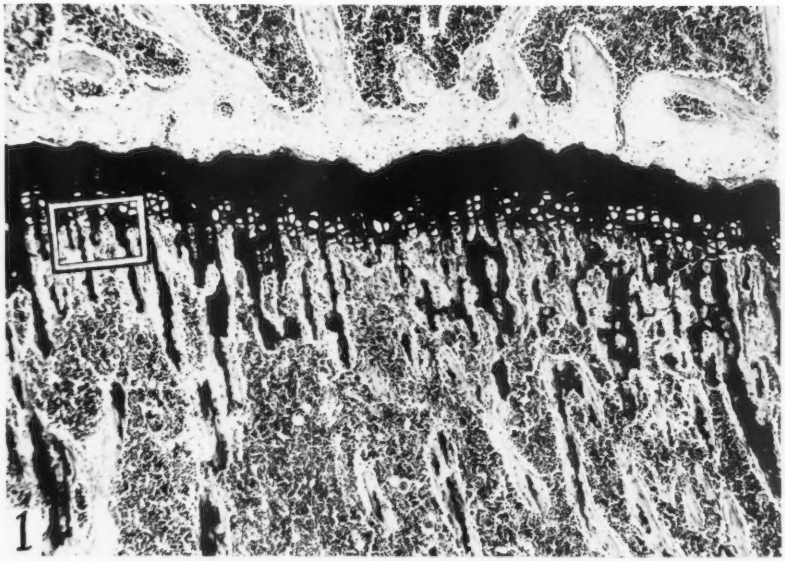
litters, in the latter case the members of each litter being divided between controls and experimental animals. Since the results of individual experiments did not demonstrate clearly any differences in chemical composition of the bones analyzed, the data were grouped for comparison and are summarized in table 1.

The combined data in table 1 show a somewhat greater mean weight of the fresh femurs from the treated animals, 79 per cent of the increase in the younger rats and 61 per cent in the older animals being accounted for as water, with smaller increases in ash and residue. The table also shows an increase in the ratio of the weight of water to the weight of the fresh bone, with only slight fluctuations in the other ratios shown. The values for *P* in the table are calculated by the method given by Dunn (21) and indicate the probability, in percentage, that the differences found are due to random sampling. The only differences showing consistently low *P* values are those referring to water content, indicating that the increases in the absolute and relative amounts of water in the bones of the rats treated with thyroid are statistically significant.

In both the younger and older animals treated with thyroid the mean of the absolute ash content is slightly increased, but not significantly so. There is a slight decrease in the amount of ash relative to residue, as measured by the percentage of ash in the dry fat-free femurs and by the ash/residue ratio, but again, especially in the older group, these differences are of doubtful significance. Taking the absolute and relative amounts of ash together, there is no evidence of decalcification in the bones of the older rats or of failure of calcification in the rapidly growing rats. From this it must be concluded that in the rat, under the conditions of these experiments, with a diet adequate in calcium, the feeding of thyroid in amounts sufficient to induce severe hyperthyroidism does not produce the specific catabolic effect upon the calcium deposits in the bones postulated by Aub, Bauer, Heath and Ropes (1).

Of the younger animals, killed at ages from 4.5 to 5 months, the tibias and femurs of 6 control and 11 experimental animals were examined histologically. At this age, in normal rats, there is active endochondral formation of bone at the junction of the shaft with the epiphyseal cartilage plate. This is evidenced by proliferation of cartilage cells in columns, degeneration of cartilage cells, cartilage removal, and formation and reconstruction of the trabeculae of spongy bone, the latter process being accompanied by the presence of osteoblasts and osteoclasts in large numbers. All of the control animals in this group correspond to this picture (fig. 1 and 3).

When growth at the junction ceases or is greatly retarded, by reason of age or otherwise, the cartilage assumes a very different appearance. It is frequently perforated, cartilage cells are few and do not proliferate or degenerate, cartilage removal ceases, or nearly so, the trabeculae of the spongiosa are resorbed, and osteoblasts are not found at all or are very few in number (fig. 2 and 4). All of the 11 younger treated animals exhibited various degrees of this retarded growth, 6 showing no evidence of growth, 4 only slight growth, and one animal slight growth in the tibia but none in the femur.



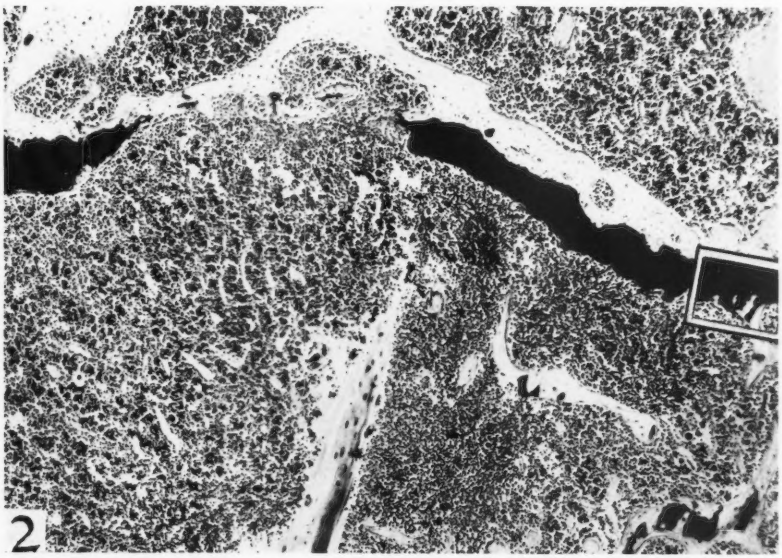


Fig. 1. Epiphyseal cartilage and zone of growth from tibia of normal rat killed at age of 4.5 months. Hematoxylin-eosin-azure, x48.

Fig. 2. Epiphyseal cartilage from tibia of litter mate of rat shown in figure 1, killed at same time. Fed 0.5 per cent thyroid for 4 weeks. Shows interruption of cartilage and absence of growth. Hematoxylin-eosin-azure, x48.

Fig. 3. Area from figure 1, indicated by rectangle in white, x480. Shows degeneration and removal of cartilage, with many osteoblasts.

Fig. 4. Area from figure 2, indicated by rectangle in white, x480. Shows quiescent cartilage, union of bone with cartilage, no osteoblasts.

Of the older rats, killed at ages of 7 and 8 months, the bones of 9 control and 9 experimental animals were examined histologically. Of the control animals 2, both killed at 8 months of age, showed the perforations in the cartilage plate and other evidences of greatly retarded growth described above, the remaining 7 showing moderately active growth. Of the 9 treated animals 7 showed extreme retardation or absence of growth, and 2 showed continuous cartilage plates with only slight growth. These two animals had received thyroid for only 4 weeks, as compared with 7 to 10 weeks for the other treated animals of the group.

DISCUSSION

In contrast with other tissues, bones at the points where endochondral ossification occurs show definite histological evidences of the activity of the growth process. Although the epiphyses of the rat do not close normally, the histological appearance changes markedly with age and decrease in rate of growth. The older rats examined were of an age at which the growth is already retarded, although most of the control animals still exhibited moderately active growth. The majority of the treated animals, as compared with the controls, showed premature retardation of growth. The findings are even more striking in the case of the younger rats, since all the treated animals showed marked retardation of growth at an age at which growth is normally very active, and 6 out of 11 showed almost complete cessation of growth.

From these observations it would appear, in confirmation of Bircher (15) that hyperthyroidism induces a premature retardation of growth in the long bones of the rat, analogous to premature closure of the epiphyses in other animals. These observations require reconciliation with the findings above reported that the bones of the treated animals were, on the average, about 5 per cent heavier than those of the controls. It would appear, as noted by Dott (16) in puppies, that there may be a temporary increase in the rate of growth of the bones, followed by premature cessation of growth. Whether in the rat, the bones of which still have the potentiality of further growth, this would result in permanent stunting, is under investigation.

SUMMARY

Chemical analysis of the bones of rats fed sufficient thyroid substance to produce severe hyperthyroidism revealed no evidence of failure of calcification in the bones of rapidly growing rats or of decalcification of the bones of older rats. It is concluded that in the rat, with a diet adequate in calcium, the feeding of thyroid does not produce a specific catabolic effect upon the calcium deposits in the bones. The wet weight of the femurs from rats fed with thyroid was greater than that of the femurs from control animals, and the difference was largely accounted for by increase in the absolute and relative amounts of water in the bones from the treated rats. There were no other significant differences in the chemical composition of the bones. There was cessation or marked retardation of growth at the epiphyseal junctions of the femurs and tibias of rats between 4.5 and 8 months of age after feeding toxic doses of thyroid for from 4 to 12 weeks. It is concluded that in the rat the feeding of thyroid may

lead to premature cessation or retardation of growth of the long bones by endochondral bone formation, possibly preceded by a period of stimulation of growth.

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THE RELATION OF ENVIRONMENTAL TEMPERATURE TO THE ACTION OF THYROXINE

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There have been few reports (1-4) dealing with the relation of environmental temperature to the tolerance to and toxicity of thyroxine, although it appears to be common knowledge that, both clinically and experimentally, the tolerance to this hormone is markedly affected by environmental temperatures. Our attention was directed toward this problem by the results of an experiment performed during a heat wave in July 1933; 4 of 5 guinea pigs that had ingested small amounts of thyroxine developed acute gastric ulcers. Prior to this time we had sacrificed 123 pigs treated with the same or larger amounts of thyroxine without finding a single lesion in the gastric mucosa. These observations were difficult to reconcile, since the guinea pigs were of the same stock and the diet and thyroxine preparations were unchanged. The only obvious differences were in the season of the year and the environmental temperature. The earlier studies had been carried out between October and April, with the temperature of the animal quarters at 20°C. ± 2.5°; the latter work was done in July with the temperature varying between 28° and 33°C.

The following experiments were designed to determine whether this increased toxicity of thyroxine could really be attributed to the high environmental temperature.

EXPERIMENTAL

(A). Confirmation experiment performed during a heat wave, July, 1933. Forty-six male guinea pigs,[1] weighing between 360 and 410 gm., were divided into two groups consisting of 16 normal control animals and 30 treated with varying quantities of thyroxine.[2] The thyroxine was ingested in doses of 0.2 mg. daily for periods varying from 1 to 5 days. (During this time and for the 2 weeks preceding, the temperature of the animal quarters fluctuated between 26° and 37°C.) Each animal was killed 48 hours after ingestion of the last dose of thyroxine and a careful examination of the stomach and small intestine was made; those areas that appeared to be abnormal were placed in Zenker's fixative and microscopic sections were prepared.

The results of this experiment are shown in table 1, and they confirm the earlier observations. None of the normal guinea pigs had gastric lesions, and neither did the pigs that ingested 0.2 and 0.4 mg. of thyroxine. Four of 8 ani-

[1] All of the guinea pigs used in this and the succeeding experiments were of the same stock and had been raised on a diet of prepared rabbit food, alfalfa hay and lettuce.

[2] Oral thyroxin, E. R. Squibb and Sons, New York City, was used: the desired quantity was suspended in water and fed to the animals with a medicine dropper.

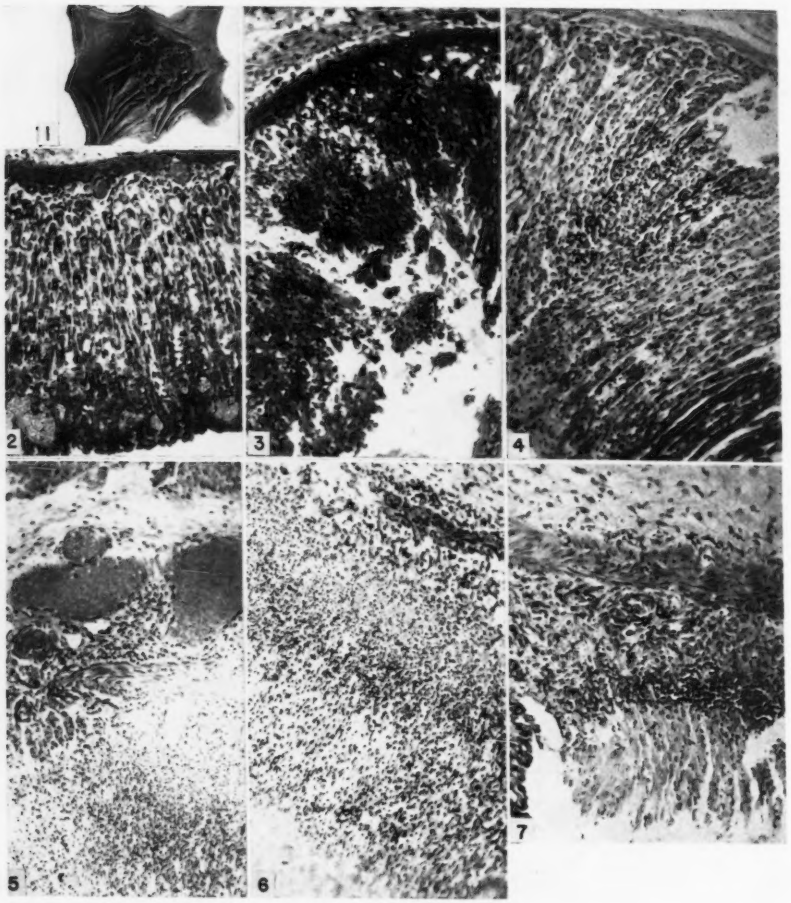

Fig. 1. Macroscopic ulcer. ⅔ actual size.
Fig. 2. Congestion in the mucosal vessels; structure of the mucosa otherwise normal. x110.
Fig. 3. Hemorrhage into the lumen of the stomach, early necrosis of the mucosa showing pycnotic nuclei and nuclear debris, a few leucocytes in the submucosa. x110.
Fig. 4. Necrosis without hemorrhage—seen in areas of the mucosa adjacent to a hemorrhagic area. x110.
Fig. 5. Congestion of vessels in the submucosa, leucocytes invading the submucosal tissues and penetrating into the necrotic mucosa. x110.
Fig. 6. Invasion of leucocytes into the neucrotic mucosa. Lumen edge is completely necrotic. x110.
Fig. 7. Leucocytes walling off the necrotic portion of the muscosa along the lumen from the less affected portion nearer the muscularis mucosae. Note the non-ulcerated mucosa at the left. x110.

mals that received 0.6 mg. and 11 of 16 that received 1 mg. had both gross and microscopic changes in the gastric mucosa. We found no lesions in the small intestine, either in these animals or in those used in the next experiment.

The lesions found in these animals and those of the succeeding experiments were similar in appearance and showed very characteristic changes. Since gastric ulcers produced in this manner have not been described heretofore, it is of interest to present briefly the important features of their gross and microscopic appearance (figures 1-7).

The lesions in the stomach were always found along the greater curvature in the lower portions of the fundus. Macroscopically, some of the ulcers were actively bleeding, whereas others were covered with clotted blood and a lightly colored filamentous material resembling fibrin. Some appeared as well-rounded, punched-out ulcers 2-4 mm. in diameter; others larger and more severe were irregular in shape; the most severe lesions, such as the one shown in figure 1, covered at least one-eighth of the entire stomach surface.

Microscopically, the lesions in different animals could be classified to represent stages of the ulcerative process. The first changes appeared to be congestion of the blood vessels supplying the mucosa (fig. 2); usually this involved the vessels in the submucosa under the muscularis mucosae and those along the lumen of the stomach, but in some instances this congestion ex-

TABLE 1. OCCURRENCE OF GASTRIC ULCERS FOLLOWING INGESTION OF THYROXINE DURING HOT WEATHER

No. of Animals in Group	Thyroxine Treatment		Results	
	Mg. Ingested Daily	No. of Days Treated	No. of Animals with Gastric Ulcers	% Animals with Gastric Ulcers
16	None	None	0	0
3	0.2	1	0	0
3	0.2	2	0	0
8	0.2	3	4	50
16	0.2	5	11	70

tended to the capillaries between the stomach glands. This stage was followed by actual hemorrhage into the mucosa—usually on the lumen edge (fig. 3).

The third stage involved the coagulation necrosis of the tissue beneath and in the area adjacent to the hemorrhage (fig. 3, 4). The parietal cells appeared to be the first affected, for their muclei became pycnotic while those of the chief cells were still normal. However, the chief cells disappeared rapidly once they were affected, the parietal cells retaining their pycnotic nuclei and cell outlines for some time. As these processes proceeded, the submucosa became oedematous.

The fourth stage was characterized by the invasion of the polymorphonuclear leucocytes and the sloughing off of the necrotic mucosa. These leucocytes accumulated first in the submucosal vessels (fig. 5), then invaded the tissues and aggregated along the submucosal side of the muscularis mucosae. They entered the mucosa, appearing to pass through the narrow septa between the glands, and penetrated about half-way or even less into the mucosal debris; there they formed almost a solid wall (fig. 6, 7).

Most of the ulcers were clear-cut; the lesions terminated abruptly, with the areas adjacent to the ulcers either normal or showing pycnotic nuclei for a very short distance (fig. 7).

(B). Hot-room experiment. The following experiment was devised to determine conclusively whether the results detailed above were due directly to the high environmental temperatures and high humidity. Two hundred and sixteen male and female guinea pigs, weighing between 280 and 350 gm. (average

331 gm.), were divided into 2 main groups. One group of 38 males and 26 females served as controls and was kept in the regular animal quarters where the temperature was 20°C. ± 2.5°, and the humidity varied with the atmospheric conditions. (These animals and those of the hot-room group were

TABLE 2. EFFECTS OF HIGH ENVIRONMENTAL TEMPERATURES AND THYROXINE TREATMENT ON BODY WEIGHT

Group	No. of Animals in Group	Sex	Animal Room Temperature	Observation Period			Thyroxine Period	
				Changes in Body Weight			Mg. Thyroxine Ingested	Changes in Weight
				After 2 Weeks	After 4 Weeks	After 6 Weeks		
				per cent	per cent	per cent		per cent
A	8	♀	20°C.	+13.0	+26.0		0	+ 3.6
B	18	♀	20°C.	+15.0	+27.0		0.6–1	− 6.7
C	19	♀	32°C.	+ 8.7			0	+ 2.0
D	9	♀	32°C.	+ 9.0	+17.0		0	+ 1.7
E	4	♀	32°C.	+ 7.0	+16.0	+22.0	0	+ 1.9
F	23	♀	32°C.	+ 8.9			0.6–1	−20.2
G	15	♀	32°C.	+ 8.0	+14.6		0.6–1	−18.7
H	10	♀	32°C.	+ 9.0	+15.1	+22.3	0.6–1	−20.5
I	14	♂	20°C.	+18.0	+34.0		0	+ 4.0
J	24	♂	20°C.	+16.6	+32.0		0.6–1	− 6.4
K	14	♂	32°C.	+15.0			0	+ 2.5
L	13	♂	32°C.	+13.0	+26.0		0	+ 2.0
M	5	♂	32°C.	+17.0	+31.0	+41.0	0	+ 1.8
N	12	♂	32°C.	+12.0			0.6–1	−12.5
O	18	♂	32°C.	+14.0	+29.0		0.6–1	−17.2
P	10	♂	32°C.	+15.0	+27.0	+44.0	0.6–1	−22.2

weighed every other day.) At the end of 4 weeks some of these animals were treated with thyroxine (0.6 — 1.0 mg.) as described previously. When the treated animals were killed, the untreated controls were also sacrificed and postmortem examinations were made.

The second group, consisting of 72 males and 80 females, was placed in a hot room, designed to duplicate the more extreme conditions of the 1933 Cincinnati summer. The temperature of this room was 32°C. ± 2° and the relative humidity was maintained between 60 and 80 per cent. The hot-room animals were divided into 3 principal groups. After 2 weeks' exposure to the hot room environment, some of the animals of *group 1* were treated with thyroxine (0.6 — 1.0 mg.); 48 hours after conclusion of treatment, both the treated and control pigs were sacrificed. *Groups 2* and *3* were treated similarly after being kept in the hot room for 4 and 6 weeks, respectively. The effects of these procedures on body weight and the incidence of gastric ulcer formation are shown in tables 2 and 3.

Exposure to a high environmental temperature and high humidity depressed the growth rate (table 2); this observation confirmed that of Ogle (5). The effect on the growth of the female animals was more marked than that on the males. In a 4-week period, the weights of the control room females increased 26 to 27 per cent, whereas in the hot room the gains were only 14.6

to 17 per cent. The weights of control-room and hot-room males increased 32 to 34 and 26 to 31 per cent, respectively.

As indicated by the loss in body weight (table 2), the reaction to thyroxine was much more severe at 32°C. than at 20°. The male guinea pigs in the control room lost 6.4 per cent of their weight following the ingestion of thyroxine; the males living at 32° lost from 12.5 to 22.2 per cent. The longer the male animals were exposed to the high temperatures the greater was the loss of weight following thyroxine treatment. This relation did not hold with the females. Those that had been kept at 32°C. for 2 and 4 weeks lost just as much weight as those kept in the hot room for 6 weeks.

As judged by the formation of gastric ulcers, the reaction to thyroxine was much more severe at 32°C. than at 20° (table 3). None of the animals kept at 20° had gastric lesions, neither the normal controls nor those treated with thyroxine. None of the control animals at 32° developed ulcers, but of 88 pigs kept at this temperature and treated with thyroxine, 43 had ulcers (49 per cent).

The female animals were the first to show an increased reaction to thyroxine treatment. After 2 weeks at 32°, ingestion of thyroxine led to formation of gastric ulcers in 12 of 23 females; with similar treatment only 2 of 12 males were affected. After 4 weeks at 32°, this sex difference disappeared; 10 of 15 females and 11 of 18 males developed ulcers. After 6 weeks in the hot room, some of the female pigs regained their natural resistance to thyroxine, whereas the males behaved like those kept at 32° for 4 weeks.

TABLE 3. EFFECTS OF HIGH ENVIRONMENTAL TEMPERATURES AND THYROXINE TREATMENT ON THE INCIDENCE OF GASTRIC ULCER

Group	No. of Animals in Group	Sex	Animal Room Temperature	Weeks Exposed to Temperature	Mg. Thyroxine Ingested	No. of Animals with Ulcers	% with Ulcers
A	8	♀	20°C.	4	0	0	0
B	18	♀	20°C.	4	0.6–1	0	0
C	19	♀	32°C.	2	0	0	0
D	9	♀	32°C.	4	0	0	0
E	4	♀	32°C.	6	0	0	0
F	23	♀	32°C.	2	0.6–1	12	56
G	15	♀	32°C.	4	0.6–1	10	67
H	10	♀	32°C.	6	0.6–1	0	0
I	14	♂	20°C.	4	0	0	0
J	24	♂	20°C.	4	0.6–1	0	0
K	14	♂	32°C.	2	0	0	0
L	13	♂	32°C.	4	0	0	0
M	5	♂	32°C.	6	0	0	0
N	12	♂	32°C.	2	0.6–1	2	17
O	18	♂	32°C.	4	0.6–1	11	61
P	10	♂	32°C.	6	0.6–1	7	70

Although the surviving females in the 6-weeks' group had no gastric lesions, the mortality of the entire group was very high. All of the animals belonging to other groups survived treatment; of the 22 '6-week' females, 12 died before the fourth day of thyroxine treatment. This might indicate that we had

one group of animals that had become adapted to the high temperatures and another that was not adapted and had lost its ability to resist thyroid stimulation.

There was no correlation between loss of body weight and the incidence of gastric ulcers. That these lesions did not result from starvation is indicated by experiments showing that guinea pigs that had been fasted until they had lost 20 to 25 per cent of their body weight did not develop ulcers.

DISCUSSION

It is not possible to offer a complete explanation for the increase in toxicity of thyroxine for animals exposed to high environmental temperatures. One may suggest, however, that the hot-room environment combined with thyroxine treatment has led to adrenal exhaustion; this has in turn induced the formation of gastric ulcers. This suggestion is supported by the following facts.

The adrenals play an important part in maintaining the normal body temperature (6-8). Exposure to cold increases suprarenal activity (9-11) and according to Mills (12) exposure to a hot moist climate decreases activity and sometimes leads to suprarenal insufficiency. A mild form of adrenal insufficiency can be changed to exhaustion by administration of thyroxine (13-17). Adrenal insufficiency, or complete absence of adrenal functioning, invariably leads to gastro-intestinal upsets and the formation of gastric or duodenal ulcers (14).

SUMMARY

These experiments have shown that thyroxine is much more toxic for guinea pigs exposed to a high environmental temperature (32°C.) and high humidity than for those kept at a lower temperature (20°C.). Gastric ulcers develop frequently when guinea pigs kept at 32° for 2, 4 and 6 weeks are treated with small doses of thyroxine. The loss of body weight is much greater after treatment at 32° than at 20°. It has been suggested that this increase in thyroxine may be due to suprarenal exhaustion.

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VARIATIONS IN THE STRUCTURE OF ADRENALS AND THYROIDS PRODUCED BY THYROXINE AND HIGH ENVIRONMENTAL TEMPERATURES[1]

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The present report concerns a study of the structural changes in the adrenals and thyroids of guinea pigs treated with thyroxine at ordinary and high environmental temperatures. This study grew out of an observation (1) that thyroxine was much more toxic (as judged by loss of weight and formation of gastric ulcers) for animals kept at 32°C. than at 20°C. It was suggested that this increased toxicity was the result of suprarenal exhaustion produced by the combination of high temperature and high humidity, and thyroxine treatment. Our present observations do not offer conclusive proof of the existence of adrenal exhaustion. The results are recorded, however, since they are so striking and have not been reported heretofore.

EXPERIMENTAL

The adrenals and thyroids were obtained from some of the 'hot-room' and 'control-room' guinea pigs whose care and treatment were described in the preceding report (1). When the animals were sacrificed, the above organs (as well as the uteri, vaginae, ovaries and portions of the livers and stomachs) were removed and fixed in Zenker's fluid, sectioned in paraffin at 10 μ and stained with hematoxylin and eosin. The adrenals and thyroids were sectioned through their longitudinal axes, and every 25th section was mounted.

The Adrenals

A). Mitotic activity. It was found necessary to study mitotic activity in the adrenal cortex. Mitoses were counted in the central sections of maximum size. This method has been used by others (2, 3) and is considered a satisfactory estimate of the activity of the entire gland.

a), Males. Controls at 20°C. Group I. The total number of mitoses per section averaged 40. Of these, 15 were in the glomerular zone and 25 in the fascicular zone; most of the latter were in the outer third of this area. Occasionally a mitotic figure was seen in the outer reticular zone or in the glomerular or fascicular connective tissue; these were not included in the averages.

Thyroxine treated at 20°C. Group J. Following the ingestion of thyroxine, there was a large increase in mitotic proliferation in almost all animals (16

[1] Read before the American Association of Anatomists, Toronto, Canada, March 1937.

out of 19). The number of mitoses varied in different individuals, from approximately two to five times as many as in the normal controls, the average being about three times as numerous. The distribution of the mitoses was altered; almost the entire number was found in the fascicular zone, scarcely any in the glomerular zone. They were not limited to the outer third of the fascicular area but were found in the deeper levels also in considerable num-

TABLE 1. MITOTIC PROLIFERATION IN ADRENAL GLANDS

Group	No. of Animals	Sex	Animal Room Temperature	Weeks Exposed to Temperature	Mg. Thyroxine Ingested	No. of Sections Counted	Average No. of Mitoses per Section		Total No. of Mitoses per Section		
							Glomerular Zone	Fascicular Zone	Minimum	Maximum	Average
A	7	♀	20°C.	4	0	26	8	41	22	72	49
B	4					8	4	63	49	79	67
	13	♀	20°C.	4	0.6–1.0	40	7	125	84	199	132
C	10	♀	32°C.	2	0	34	3	12	5	38	15
D	5	♀	32°C.	4	0	11	6	12	8	27	18
E	3					12	10	9	11	28	19
	2	♀	32°C.	6	0	8	35	15	33	78	50
F	18					75	3	44	6	97	47
	1	♀	32°C.	2	0.6–1.0	3	3	161	142	194	164
G	10					27	4	21	12	66	25
	2	♀	32°C.	4	0.6–1.0	7	9	136	76	243	145
H	1					3	8	158	143	179	166
	1	♀	32°C.	6	0.6–1.0	2	18	591	517	665	609
I	9	♂	20°C.	4	0	37	15	25	24	63	40
J	3					12	4	48	20	77	52
	16	♂	20°C.	4	0.6–1.0	48	6	109	70	190	115
L	5	♂	32°C.	4	0	15	4	12	8	29	16
M	5	♂	32°C.	6	0	12	12	30	24	70	42
O	2					4	1	71	64	66	65
	7	♂	32°C.	4	0.6–1.0	26	2	129	91	221	131
P	5					21	2	47	23	83	49
	2	♂	32°C.	6	0.6–1.0	12	1	130	91	220	131

bers. More mitotic figures were seen in the connective tissue, as many as 8 per section. These findings confirm and extend Deanesly's qualitative observations in mice (4).

Three of the 19 animals showed little increase in number of mitoses; however, the distribution showed the thyroxine effect even though the total numbers were not increased.

Controls at 32° C. Groups L and M. After 4 weeks' exposure to a temperature of 32°C. (*group L*) there was a marked and uniform suppression of

mitotic activity. The total number of mitoses averaged only 16 per section, and one-fourth of these were in the glomerular zone. As the animals continued to stay in the hot room they apparently became adjusted to the high temperatures, for at the end of 6 weeks (*group M*) mitotic proliferation was similar to that of the 20°C. controls, with a similar distribution in glomerular and fascicular zones.

Thyroxine treated at 32°C. Groups O and P. Although adrenal activity was suppressed after 4 weeks in the hot room, the ability to react to thyroxine was not lost (*group O*). In 8 of 9 animals, the response was similar in nature and extent to that in animals kept at 20°. Since the mitotic activity was much less in the untreated animals at 32° than in the group at 20°, the response to thyroxine in the hot-room group was actually much greater than in the group kept at ordinary temperature; on the average the mitoses were 8 times as numerous as in the adrenals of untreated animals. Almost all the mitoses appeared in the fascicular zone.

After 6 weeks in the hot room, the adrenals of the majority of animals (*group P*) could not respond at all to thyroxine treatment, and those that could respond did so to a lesser degree than the group above. Five animals did not respond at all; the average number of mitoses was almost identical with that of the untreated animals. The adrenals of 2 of the 7 guinea pigs had as many mitoses as those which had been treated after 4 weeks in the hot room. Again, the distribution of mitoses showed the thyroxine effect in all the adrenals—nearly all in the fascicular zone—regardless of whether the total number of mitoses had increased.

b), Females. Controls at 20°C. Group A. The average number of mitoses per section was slightly higher than that in the males, 49 and 40 respectively.[2] The individual variations were greater in the females; this was particularly true in the glomerular zone where the number of mitoses was only about 17 per cent of the total as compared with 37 per cent in the males. Animals were too few in number to permit correlating individual variations with stages of the estrous cycle, but according to Schmeckebier (3) no relation exists.

Thyroxine treated at 20°C. Group B. The response to thyroxine feeding was identical to that obtained with the males. Thirteen of 17 animals responded greatly, so that the average number of mitoses was 132 per section. Four animals responded very slightly or not at all but, as in the males, the mitoses showed the thyroxine distribution.

Controls at 32°C. Groups C, D and E. There was a marked and uniform suppression of mitotic activity, which appeared in the adrenals of animals kept in the hot room for only two weeks (*group C*) and persisted for at least 4 weeks (*group D*). In *group C* the distribution of mitoses was the same as in *group A*, but in *group D* the proportion of mitoses in the glomerular zone had increased somewhat.

[2] Our mitotic counts in the adrenal cortex of normal males and females were higher than those reported by others. Schmeckebier (3) found an average of 1.5 figures per section cut at 6 μ, Hoerr (2) 2 to 8 per section cut at 3 to 4 μ. These counts were also on central sections, in animals weighing approximately as much as ours. Temperature differences may be partially responsible for this variation. Thickness of section is one factor (actual counts on successive sections showed an average of 25 per cent more mitotic figures in sections cut at 10 μ than at 7μ), but it can not entirely explain the differences.

The mitotic activity in the male adrenals returned to the normal 20° level after 6 weeks in the hot room; not all the females were able to accomplish this (*group E*). Three animals remained at the same level as at 2 and 4 weeks; 2 returned to the normal level. In all 5 animals, the distribution of mitoses was reversed. More mitotic figures were found in the glomerular than in the fascicular zone; this reversal was most marked in those animals in which the number of mitoses had returned to normal. This was the only instance in these experiments in which this particular distribution occurred; Schmeckebier (3) stated that this occurred in many of her normal guinea pigs, but Hoerr (2) rarely found mitoses in the glomerular zone.

Thyroxine-treated at 32°C. Groups F, G and H. The 'hot-room' females did not respond to thyroxine as well as did the males. After 2 weeks at 32°, only 1 of 19 pigs responded with greatly increased mitotic activity (*group F*); in the other 18 animals the response was either relatively small or entirely lacking. Similarly after 4 weeks, only 2 of 12 animals responded markedly; in the other 10 animals there was very little stimulation (*group G*). After 6 weeks in the hot room a large proportion of the females was unable to withstand the thyroxine treatment and died before it was completed. The mitotic response was tremendous in the 2 survivors studied (*group H*)—166 mitoses per section in one animal and 609 in the other.

In the 2-week group described above, there were present in most adrenals many nuclei which looked as though they were about to become prophases, but were not yet definite enough to be classed as such. This might indicate a delayed response to thyroxine in this group, or the beginning of stimulation with inability to complete it.

B). Size. The adrenals of the normal controls varied so greatly in size and weight that the averages were of little significance. This variation occurred without apparent relation to the mitotic activity of the gland. The size of the adrenal could not be correlated with body weight, since all of our animals were in a very limited weight group. This observation confirms the more detailed study of Kosaka (5).

The adrenals of the animals kept at 32° for 2 weeks were as large as those of the normal controls, even though mitotic activity was suppressed. After 4 weeks at 32° the adrenals were definitely smaller than the normals; this small size persisted in those animals that did not regain normal mitotic activity in 6 weeks; these latter were essentially normal in size.

The adrenals of animals receiving thyroxine were larger than those of the untreated controls. This was especially noticeable in the groups kept at 32°.

C). Distribution of the lipoid material. a), Microliposomes. In the adrenals of normal males kept at 20°C., the lipoid-containing portion of the fascicular zone occupied the outer one-half or more of the cortex, merging rather abruptly into the short transition region whose cells contained scattered microliposomes. Although generally similar in the females, the lipoid-containing layer was more variable in size.

In the adrenals of both males and females kept at 32° for 2 and 4 weeks, the lipoid-containing zone was shorter, occupying not more than the outer

third of the cortex. The transition zone was widened, occupying another third. In the glands of the females the microliposomes were larger and more numerous than in the males. After 6 weeks at 32°, some glands were still like those at 4 weeks, whereas others were like the controls. Thyroxine treatment produced no significant change in either the 20° or 32° groups.

b), Macroliposomes. Macroliposomes were scarce or absent in all male glands under all conditions. The number present in the control females at 20° was variable. In half the glands there were a few macroliposomes scattered through the fascicular zone; the other half contained abundant numbers located all through the fascicular zone, with some tendency to concentration under the glomerular zone, but even extending into the reticular zone. This confirmed Zalesky's findings (6). A similar distribution occurred in each of the 'hot-room' control groups.

After 2 weeks at 32°C., thyroxine treatment increased the number of macroliposomes (in the females only) and concentrated them near the reticular zone. This did not occur in any of the other groups.

D). Reticular zone. This zone was widest in the 'hot-room' animals. There was also a wide transition zone in this group. 'Dark' cells were numerous in all groups, but were more abundant in the 32° groups; the entire widened zone was filled with them. The width of the reticular zone and the number of 'dark' cells were no greater in thyroxine treated animals than in the corresponding controls.

E). Medulla. If any structural changes were present, they were not apparent. Some partially degranulated cells were present in all groups and were probably due to imperfect fixation. Mitoses were rare. No constant vascular changes were seen. Volume changes were not studied.

F). Effects of underfeeding. The question arose whether the changes in mitotic activity were due to the loss of weight following thyroxine treatment, for it has been reported (3, 7) that underfeeding causes an increase in mitotic divisions in the cortex. The fact that the degree of mitotic activity did not parallel the weight loss ruled out this possibility. Nevertheless, a group of animals maintained at 20°C. was underfed so that weight was lost at the same rate as in the thyroxine treated animals.

Mitotic activity was but little affected by weight losses of 6 per cent (this was the average weight loss of 20°C. animals treated with thyroxine). With greater weight losses, 13 to 26 per cent, mitotic activity decreased. Thus, the greatly increased number of mitoses following ingestion of thyroxine could not have been due to the loss of weight.

Furthermore, with a weight loss of 13 per cent or more, structural changes occurred which were not met in any of the preceding experiments. The reticular zone occupied half the cortex; numerous 'dark' cells with shrunken or pycnotic nuclei were present throughout, extending up to the boundary of the fascicular zone. The fascicular zone, already much smaller than in the normal controls, showed two distinct regions—a narrow outer one just under the glomerulosa, whose cells were large and filled with large uniform microliposomes; and an inner zone of cells with fine, indistinct microliposomes, presenting a granular

appearance. The macroliposomes were quite numerous and were found in the fascicular zone.

The Thyroids

No striking changes occurred in the thyroid glands which have not already been described by others. The control animals at 20° had glands whose follicles were lined by plump cuboidal cells; the colloid was moderately hard, somewhat retracted in the larger follicles but filled the smaller peripheral ones. Capillaries were prominent.

Thyroid activity was suppressed in the hot room. After 2 weeks the epithelium was somewhat flattened and the colloid was slightly retracted; some glands were vascular. In 4 weeks the epithelium was definitely low, the colloid was hard and retracted and capillaries were inconspicuous. In 6 weeks the epithelium was still low, but the acini were filled and the capillaries were again prominent.

Following thyroxine treatments, the epithelium was also low or flattened, the colloid filled the acini and capillaries were not congested. These changes were not altered by exposure to the 'hot room.'

DISCUSSION

Many investigators have observed adrenal enlargement following the ingestion of thyroid gland or thyroxine at ordinary temperatures (8); this enlargement has involved the cortex. Our data are in accord with this earlier work; the increased mitotic activity, found in both males and females after ingestion of thyroxine, offers even more striking evidence of cortical hyperactivity than the increase in adrenal size.

The initial exposure to heat suppressed cortical activity in both males and females; after a prolonged exposure an interesting sex difference appeared. The untreated males were able to overcome the depressing effects of heat and in 6 weeks the cortical activity returned to normal; the females could not do this. The same sex difference occurred in thyroxine-treated animals. When males were treated after 4 weeks at 32°C., cortical activity was increased considerably; after 6 weeks the increase was slight. Females kept at 32° for only two weeks responded very little and even less after 4 weeks, but in 6 weeks the response was tremendous in those animals that survived.

These observations may explain the increased toxicity of thyroxine at higher environmental temperatures. It has been shown by Oehme and his coworkers (9) that cortical hormone inhibits certain physiological actions of thyroxine. Our work and that of others indicates that the adrenal cortex of normal animals proliferates in response to an excess of thyroxine. This property is at least partially lost after exposure to heat. Proof that this is the correct explanation must await evidence that mitotic proliferation parallels the rate of cortical hormone formation.

SUMMARY

Male and female guinea pigs were exposed to a moderate room temperature (20°C.), and to 32°C. and a high humidity, for 2 to 6 weeks. Adrenals

and thyroids were studied after ingestion of thyroxine and in untreated animals. Adrenals varied chiefly in the degree of mitotic activity of the cortex and the distribution of the mitoses. In control males and females most mitoses were in the fascicular zone, but a number were found also in the glomerular zone. Following ingestion of thyroxine at 20°, the majority of adrenals of both sexes showed a great increase in the number of mitoses and a redistribution—nearly all were in the fascicular zone.

Exposure to 32° for 2 to 4 weeks suppressed mitotic activity in both sexes. Males, but not all females, were able to return to the normal condition after 6 weeks. Following thyroxine ingestion, after 4 weeks of heat, most males were able to respond as well or better than control-room males; after 6 weeks the majority did not respond. Females were less able to stand the thyroxine treatment; there was little or no response in the majority at any time. Thyroid activity was suppressed in the hot room but returned to normal in some animals after 6 weeks. Thyroxine did not further alter the picture.

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A SYNDROME OF INFANTILISM, CONGENITAL WEBBED NECK, AND CUBITUS VALGUS[1]

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The triad, infantilism, webbing of the skin of the neck, and deformity of the elbow (cubitus valgus), occurring in the same individual is unusual, and, to my knowledge, has not been previously reported. It is sufficiently interesting to warrant this report, although the individual signs are perhaps not uncommon. Short neck, due to absence of the cervical spine, was first described by Klippel and Feil (1) in 1912. Only about 40 such cases have been reported to date, and these mostly in the French and German literature.

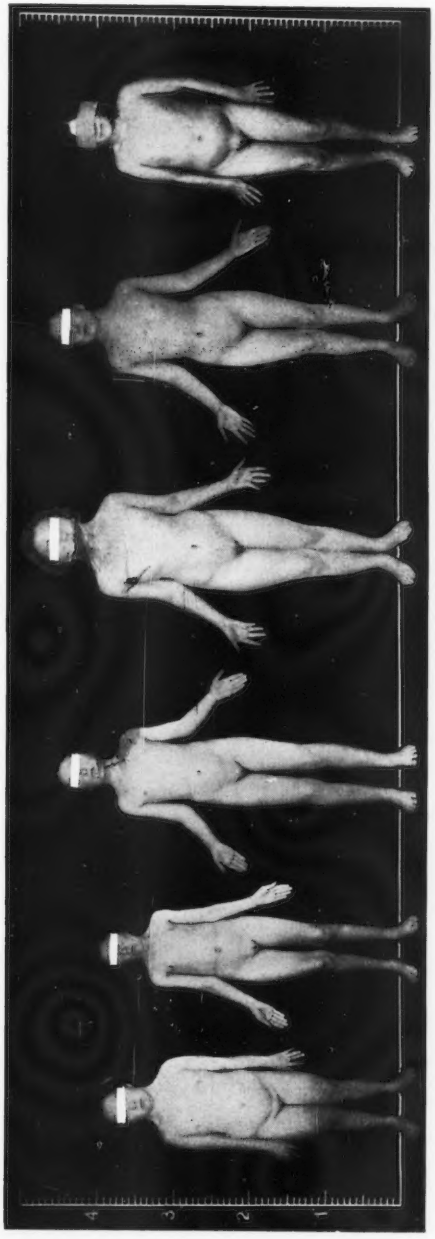
A similar condition (pseudo-Klippel-Feil syndrome) (2) has been reported, in which there was a numerical variation in, and more or less complete fusion of, the cervical vertebrae. Webbing of the neck was first described by Kobylinski (3) in 1882, and its relation to congenital short neck was suggested by Darchter (4) in 1922. Frawley (5) states that webbing is the most uncommon feature of congenital short neck, the most usual being the absence or fusing of the cervical vertebrae.

Sprengel's deformity (congenital elevation of the scapulae and other conditions, such as traumatic or spontaneous dislocation of the atlas on the axis (Frissell's disease), cervical Potts' disease, and sometimes torticollis) simulates congenital webbed neck. The classic objective signs of Klippel-Feil syndrome, as given by Bauman (6), are: *a)*, absence or shortening of the neck; *b)*, lowering of the hair-line on the back of the neck; *c)*, limitation of motion. Other signs occurring in a certain percentage of cases are: *a)*, torticollis, *b)*, mirror movement, *c)*, facial asymmetry, *d)*, dorsal scoliosis, and other deformities accompanied by difficulty in breathing or swallowing and shortness of breath.

The group of patients presented in this report cannot be classified under any of the above syndromes, inasmuch as the shortening of the neck is merely apparent, due to the webbing, and not real. There is no absence or fusion of the cervical spine in any of these patients. There is some lowering of the hair-line on the back of the neck, but no marked limitation of motion. Torticollis, mirror movements, facial asymmetry, and other signs and symptoms, such as difficulty in breathing or swallowing, shortness of breath, etc., are not present. There is no mental retardation. Deformity of the elbow, consisting of an increase in the carrying angle, or cubitus valgus, is constantly present.

All of my patients present osseous and sexual retardation similar to that

[1] Read before the 22d Annual Meeting of The Association for the Study of Internal Secretions, San Francisco, California, June 13-14, 1938.



Case 3 Case 7 Case 6 Case 2 Case 1 Case 5

Fig. 1. Patients illustrating the syndrome of infantilism, webbed neck, and cubitus valgus.

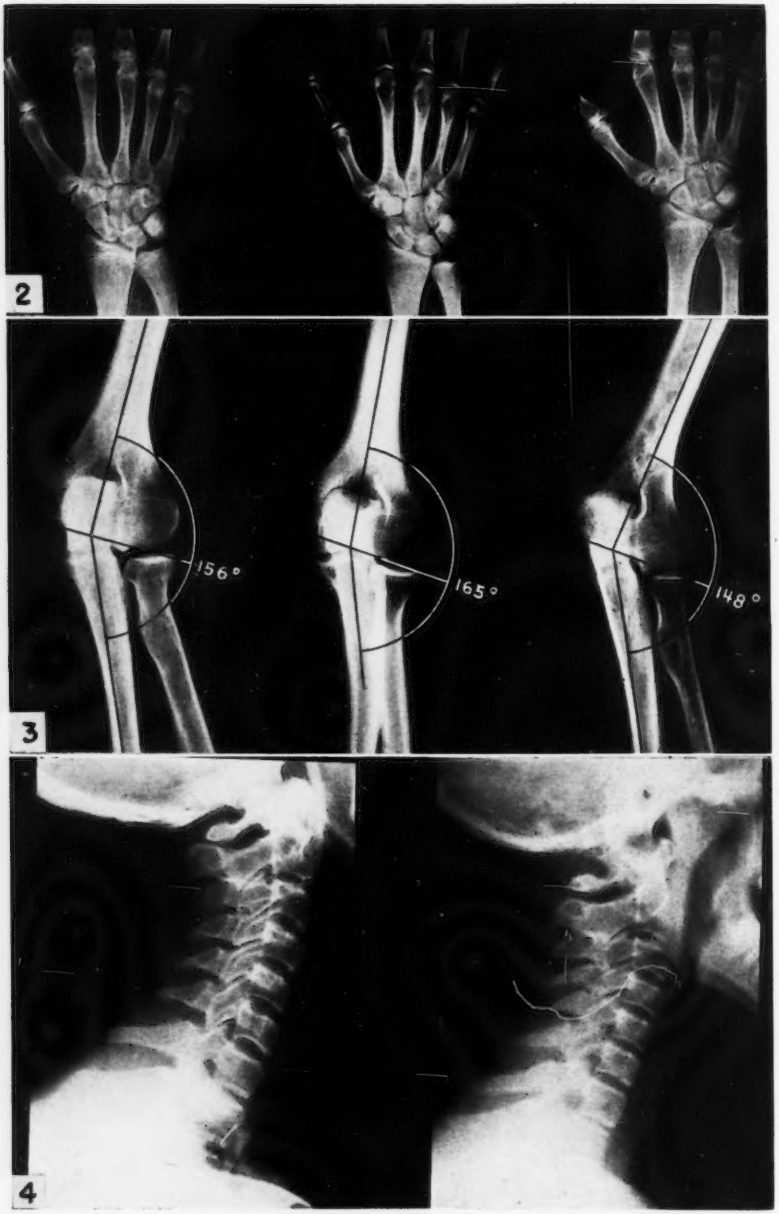


Fig. 2. *Case 1* (left) and *case 2* (right) compared with normal of same age. Note retardation of epiphyseal union.

Fig. 3. *Case 1* (left) and *case 2* (right) compared with normal of same age. Note increased carrying angle in both patients.

Fig. 4. *Case 1* (left) and *case 2* (right) showing normal cervical spine.

associated with hypoantuitarism, or the Lorain-Levi type of dwarfism. Funke (7) reported a case of 'pterygium colli,' which was somewhat similar to mine, in that the patient, aged 15 years, was retarded in growth and sexual development; however, no reference was made to any osseous deformaties.

CASE REPORT

Case 1. D. G., a female, aged 16 years 3 months, was first seen by me on March 28, 1932. She walked, talked, and teethed at the normal age. Her stature has always been less than that of her playmates of the same years. The patient showed somatic and sexual underdevelopment of the Lorain-Levi type. Her height was 53 in. (average 63.35 in.), and weight 78.25 lb. (average 118 lb.). There was no evidence of any secondary sex characters. Her breasts were quite masculine, and no glandular tissue was palpable.

Gynecological examination by Dr. J. B. Eskridge revealed greatly delayed development of the labia; the vagina barely admitted the index finger and was about 1.6 in. deep. The mucosa was dry and of the infantile type. The cervix was about the size of a pea, and there was an apparent congenital absence of the uterus and ovaries.

There was an internal squint of the right eye. The neck appeared very short, and there was a wing-like fold of skin extending from the mastoid region almost to the acromion. The hair-line was very low on the back of the neck. There was a marked deformity of the carrying angle of the elbow. No other abnormal physical findings were elicited.

Laboratory. The erythrocyte count showed a very moderate secondary anemia. The blood serology and chemistry gave normal reactions. The B.M.R. was +8 per cent. Roentgenograms of the skull and sella, cervical vertebrae, and hips indicated normal development. A retardation of the epiphyseal union on the metacarpals and phalanges was noted. The carrying angle at the elbow measured 156° (fig. 3).

Injections of the anterior-pituitary growth fraction, antuitrin-G, were begun, and over a period of 19 months she received 1600 cc. of this substance. Her height increased 1.75 in. (to 54.75 in.) during this period. Shortly after discontinuance of the growth hormone the patient left the city, and nothing further was heard from her until March, 1937. At that time, her height was 56.50 in. and her weight 88 lb. During the 3-year interval without treatment she had grown 1.75 in. and had gained 3 lb. There had been little, if any, change in her general appearance. The breasts and nipples continued very infantile, and there was no apparent change in her internal or external genitals. There was slightly increased hair growth over the lumbo-sacral region and over both trapezi down to the level of the scapulae. A great number of pigmented moles were noted over the trunk, with a few on the face, and there was some slight hair growth on the upper lip.

At this time, injections of 2½cc. anterior-pituitary gonadotropic (25 U)[2] were administered 3 times weekly and continued for 30 days. Then, injections of 1 cc. daily were begun and continued without interruption until Oct. 11, 1937. At this time, the gynecologist reported a scant growth of hair on the mons, with rather abundant growth over the labia majora; the vagina was much larger than on the previous examination, admitting one finger very easily, and if the vulvovaginal orifice were large enough, two fingers could be admitted. The vaginal length was

[2] Antuitrin-G, antuitrin-gonadotropic, and theelin generously supplied by Parke, Davis & Co.; progynon-B by Schering Corp.; anterior-pituitary sex hormone, and maturity factor by Ayerst, McKenna & Harrison.

3.5 in., rugae normal and moist. The probe passed through the cervical canal to a depth of 2.2 in. With the probe in the cervix, another attempt was made to locate the uterus, but the organ or adnexa could not be palpated. It was thought the probe passed through the Müllerian duct. The cervix was 0.75 cm. in diameter, very rudimentary and short. There was no evidence of development of the nipples or breasts. It is interesting to note that there had been a definite growth in the size and depth of the vagina, to the point that it would be possible for her to marry and carry on normal marital relations.

Anterior-pituitary sex hormone (330 U maturity factor, and 75 U thyrotropic), 1 cc. daily, plus 40,000 U of theelin per week were given until Feb. 1938, at which time it was noted that the pubic hair had increased; a small uterus about the size of a pecan was palpated, and glandular tissue could be palpated in the breasts. Her weight was 94 lb., and height 57 in.

Because of the multiple injections and the fact that the buttocks were becoming quite infiltrated, it was decided to discontinue the daily injections of anterior-pituitary sex hormone but continue the 10,000 U of theelin 4 times weekly. Following the injection of a total of 170,000 U of theelin during the next four weeks, dosage was increased to 50,000 U of progynon-B 3 times weekly. Following the 6th injection, an apparently normal menstrual flow began and lasted for 3 days. She stated that the flow was moderate in amount and no pain was experienced. The injections were discontinued during this week of flow and then continued as before. Following 7 injections, or a total of 350,000 U of progynon-B, and after a rest period of 7 days, a second menstrual flow began, with an average amount of discharge for 2½ days, then scant for 3 days. No pain was encountered. Whether or not the two periods of bleeding were the true menstrual type or result of the large doses of estrin I am unable to say.

To date, she has received a total of 17 injections (850,000 U) of progynon-B. Her height is 57 in., and weight 99 lb. Pubic hair is increasing. Labia minora are still small, but showing definite evidence of development. Labia majora are small, but appear normal. The vagina easily admits one finger and is about 2.4 in. long and considered normal in size and shape for a person of this age. The cervix is about 1 inch across and much larger than on previous examination. A fundus uteri can be definitely palpated, being 1 inch across and about 1 inch thick, and approximately 2 in. long. The cervix forms about one-half of the uterus. Glandular tissue is now palpable in both breasts, and they are assuming an adolescent contour.

Case 2. O. D. L., a female, aged 18 years, was first seen on Sept. 7, 1934, because of statural underdevelopment and non-appearance of the secondary sex characters. Her height was 55 in. (average 65 in.), weight 86 lb. (average 126 lb.). There was nothing unusual in her history during infancy or childhood, with the exception that she had always been smaller than other children. She had always made excellent grades in school and at this time was taking a secretarial course. There had never been any serious illnesses. One brother, aged 15 years, and one sister, aged 25 years, father and mother, are all of average height and weight. Her general appearance was that of an anterior-pituitary dwarf of the Lorain-Levi type. There was complete absence of any secondary sexual characteristics. The breasts were flat, with no glandular tissue palpable, and the nipples were rudimentary. The neck appeared quite short, and on either side the skin appeared as a wing-like fold, extending from the mastoid almost to the acromion process. There was an abundance of black hair on the scalp, extending down the back of the neck to a point slightly below the first dorsal vertebra. The remainder of the body was devoid of hair, with the

exception of very scant growth in the axillae and a few scattered over the pubis. The facial features were small, clear-cut, and there was no obvious asymmetry of the face. The eye muscles were intact. There was a marked increase of the carrying angle of the elbow.

Vaginal examination by Dr. Eskridge disclosed the labia markedly underdeveloped, and an absence of hair on the mons, with very scant growth over the labia. The vagina was too small for digital examination, and the uterus and adnexa could not be palpated. A small structure about the size of a pea was believed to be a rudimentary cervix.

Laboratory. The blood cytology, serology, and chemistry were all within normal limits. B.M.R. was +7 per cent. Chemical and microscopical examination of the urine revealed no abnormal constituents. Roentgenograms showed retardation of epiphyseal union on metacarpals and phalanges; very marked cubitus valgus; small and shallow sella; a slight osteoporosis of the spinal column; and separate ossification centers on both transverse processes of the first thoracic vertebra. The carrying angle of the elbow measured 148° (fig. 3).

Anterior-pituitary growth hormone, 2 cc. 3 times weekly, was prescribed, and injections were continued for approximately 5 months without any appreciable increase in her height. In Oct., 1936, injections of 1 cc. maturity factor (200 U) 3 times weekly were begun and continued until April 1, 1937. Examination at this time revealed increased growth of hair on the labia and scanty growth on the mons. The labia minora were rudimentary and small. The vagina barely admitted the index finger, but the rugae were normal and moist. The cervix, about 1 cm. in diameter, did not admit a probe. The uterus and adnexa were not palpable.

Injections of antuitrin gonadotropic, 2½ cc. 3 times weekly, were then begun, and over a period of 7 months she received 70 injections, or a total of 4,375 U. At the end of the treatment period no development of the breast tissue was evident. However, the pubic hair was becoming more abundant. The vaginal canal was moist and admitted the index finger very comfortably. The uterus and ovaries still could not be palpated. At this time, her weight was 85 lb., and height 55.75 in.

Anterior-pituitary sex hormone, 2 cc. twice weekly, was begun Oct. 11, 1937, and continued to date (76 injections). At the present time her height is 56 in., and weight 91 lb. There is definite development of the labia majora. Introitus admits one finger snugly. The cervix is palpable, although still infantile, but the uterus has not yet shown signs of developing.

Case 3. V. C., a female, aged 15 years 4 months, was a normal full-term baby who had her first tooth at 9 months. She walked and talked at 13 months. Her statural development was apparently normal until the age of 9 years, but she had matured very little since then. There was no mental retardation.

The patient's general somatic development was definitely retarded, although proportional. Her height was 50 in. (average 62.27 in.), and weight 74 lb. (average 115 lb.). The upper, lower, and half-span measurements were 25 in., respectively. The neck was short and wide, and the hair-line low posteriorly. There was a moderate increase in the carrying angle of the elbow, and an internal squint of the right eye. There was no obesity. The extremities were quite acrocyanotic. The skin and hair were of normal texture. A slight hypertrichosis of the forearms was present. No breast development was evident. There were a few scattered hairs over the mons veneris. Rectal examination revealed a very infantile uterus, but no adnexa were palpated.

Laboratory. The blood count, Wassermann reaction, and chemical examinations,

including serum calcium and phosphorus determinations, were all within normal limits. The urinalysis was normal. B.M.R. was +9 per cent. Roentgenograms of the wrists, shoulder, knee, skull and sella turcica, cervical spine, and elbows revealed normal osseous progress.

She received anterior-pituitary growth hormone (10 R.U.), 3 cc. subcutaneously every other day over a period of three months, with an increase in her height of 1.3 inches (theoretical normal increment 0.15 in.). Further treatment during the following 6 months, interspersed with rest periods, resulted in no further growth. In a letter received March, 1938, the mother stated that the patient's height was the same as when the growth hormone was discontinued 2½ years previously, and that sexually she remained quite infantile.

Case 4. M. B., a female, aged 16 years, consulted me first in Sept., 1934, because of dwarfism and sexual underdevelopment. Her height was 53.75 in. (average 65 in.), and weight 69 lb. (average 128 lb.). She was the second child in a family of 5, and had always been smaller than other children of her age. Examination revealed a definitely underheight and undernourished girl, with no visible evidence of secondary sex development. The neck was short and somewhat broad at the base, although there were no marked folds of skin comparable to the cases reported above. The facial features were very delicate. There was a normal amount of brown hair on the scalp, but none in the axillae or on the pubis. There was a marked increase in the carrying angle of the elbow, as observed in the other individuals of this series.

A scanty growth of hair appeared on the labia majora only. The labia minora were rudimentary, and the vagina would not admit the index finger. On rectal examination the broad ligament was found to be indistinct. There was a small mass about 0.5 in. in diameter, which was interpreted to be the cervix. The corpus uteri and ovaries were not palpable.

Roentgenograms of the skull showed an increased deposition of calcium along the coronal sutures. The sella turcica was well within normal limits, and there was no erosion of the clinoid processes. A rather marked degree of demineralization of the carpals and metacarpals, and the distal end of the ulnae and radii was demonstrated. The epiphyseal lines of the ulnae and radii and proximal ends of the metacarpals were quite visible, showing some delay in the union of the epiphyses. The cervical vertebrae were of normal number and appearance. No other laboratory procedures were recorded. No treatment.

Case 5. M. T., a female, aged 23 years, was referred to the Endocrine Clinic, University Hospital, Feb., 1935, because she was under height and had not developed sexually. She stated that she stopped growing at the age of 8 years, and was considerably disturbed because of the fact that her associates treated her as a little girl and because she feared she would never be able to marry and have a home. Her height was 52.25 in. (average 65 in.), and weight 76.75 lb. (average 130 lb.). She presented a typical picture of somatic and genital retardation with a rather oldish face. She had a very short, broad neck, with folds of skin extending from the mastoid to a point just proximally to the acromion. The hair-line extended well down the back of the neck. There was a very small amount of axillary hair, and none on the pubis. The breasts were flat with underdevelopment of the nipples. There was a marked increase in the carrying angle of the elbow.

The vaginal examination revealed very small, underdeveloped labia. The vagina admitted one finger. The uterus was about the size of that of a 6 or 7-year-old child. The ovaries could not be palpated.

Roentgenograms of the skull and sinuses revealed no apparent pathology. The

epiphyses of the femurs were demonstrated. A considerable lack of calcium in the bones of the pelvis and thighs, and a demineralization in the distal end of the humerus and proximal ends of the ulna was evident, with an apparent beginning cystic degeneration in the right and left ulna. Anteroposterior and lateral views of the cervical spine revealed a spinabifida of the first cervical vertebra. The bodies of the vertebrae, however, were of normal size and position. Further laboratory investigation was refused by the patient. No treatment.

Case 6. M. A. S., a female, aged 15 years, was seen in the out-patient department of the Endocrine Clinic, University Hospital, March 1, 1937, because of her dwarfism and sexual underdevelopment. She was 51.75 in. in height (average 62.27 in.), and weighed 69 lb. (average 115 lb.). She was a normal full-term baby, and walked, talked, and teethed at the usual age, but had always grown very slowly. She was mentally alert and ahead of her class in school—an honor student.

Physical examination revealed evident infantilism, with very short neck and wing-like folds of skin extending from the mastoid region to the acromion. The hair-line was very low. There was no axillary or pubic hair. The increased carrying angle of the elbow was quite marked. Vaginal examination could not be made because of the infantile vagina. Rectal examination revealed a minute mass which was interpreted as an infantile uterus. The ovaries were not palpable.

Laboratory. The blood cytology, serology, and chemistry were all within normal limits. Roentgenograms of the cervical spine and pelvis, including both hip joints, were normal. The radial deviation of both elbows was quite prominent. The radii appeared shorter than normal, and the heads were deformed. The right wrist showed an absence of the distal epiphyses of the radius, and the carpal bones were atrophic. There was also a flattening and partial sclerosis of the epiyhpses of the left ulna and radius. These observations indicated a growth disturbance; however, it was not determined whether this was of nutritional or endocrine origin. Chondrodysplasia did not seem likely. No treatment was given.

Case 7. M. B., a female, aged 16 years, was referred to the Endocrine Clinic, University Hospital, Dec. 27, 1935, because of her short stature and lack of sexual development. She was 48.50 in. in height (average 63.35 in.), and weighed 46.50 lb. (average 118 lb.). Her twin sister, who was with her, was 63 in. in height, and weighed 125 lb. The history was rather interesting, in that the patient weighed 2½ lb. at birth, while her twin weighed 5 lb. Her weight at one year was 5 lb., and that of her sister 15 lb. She had one brother and one other sister of average height and weight for their age. The parents stated that she had gained none in height or weight during the past 5 or 6 years, and that she grew very slowly before that time. She presented a rather typical picture of infantilism with congenital webbed neck and cubitus valgus. Her sister was normally developed sexually, and normal menarche was established at the age of 12 years.

Routine laboratory examinations gave essentially normal findings. Roentgenograms of the skull and sella turcica appeared normal, except for some slight demineralization. X-rays of the extremities revealed normal osseous progress, but evidence of osteoporosis. The cervical vertebrae were normal in number and appearance. No treatment was given.

SUMMARY

Infantilism with webbing of the neck and deformity of the elbow (cubitus valgus), occurring in the same individual is extremely rare, and, to the author's knowledge, has not been previously described. This unusual phenomenon was

observed exclusively in seven female patients, aged 15 to 23 years. Among the characteristic signs were retardation in growth and sexual underdevelopment. Webbing of the skin of the neck was slight to marked. Absence or fusion of the cervical vertebrae was not demonstrated, and the shortening of the neck was merely apparent, due to the webbing, and not real. The posterior hair margin extended well down on the neck. Deformity of the elbow, consisting of an increase in the carrying angle, or cubitus valgus, was constantly present. Movements of the head and arms were not hindered. Fascial asymmetry, dorsal scoliosis, and other deformities, mirror movement, difficulty in breathing and swallowing, shortness of breath, or mental retardation were not present in this group of patients.

Laboratory examinations of the blood and urine showed findings that were entirely within normal limits. Roentgenograms of the skull, cervical spine, elbow, wrist, and pelvis showed no abnormalities with the exception of demineralization and evidence of delayed union of the epiphyses in 6 cases. Treatment with pituitary growth hormones has been unsatisfactory. There was definite genital development following administration of the anterior pituitary gonadotropic hormone in the two cases treated.

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Addendum. Since this paper was written three additional cases have been observed making a total of ten patients to date.

INFLUENCE OF AVITAMINOSES ON WEIGHTS OF ENDOCRINE GLANDS[1]

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When investigations were begun several years ago on the histopathology of tissues, including those of the endocrine glands, in deficiency diseases it was thought that the weights of the endocrine glands might prove helpful in the interpretations of the microscopic observations. Later on, as this department began to pursue biochemical investigations on the endocrines in avitaminoses, the accumulation of a large amount of such additional data became available. The only definite correlation that could be made between endocrine weights and microscopic changes was found in the case of the thymus. The marked atrophy of that gland in vitamin-B_1 deficiency was evidenced histologically by connective tissue replacement. From the sections examined in vitamin-B_2 deficiency there was evidence of the thymic involution, in the presence of fat and fibrous connective tissue (1).

Since, in the majority of the deficiency diseases, the progressive development of the avitaminotic state is accompanied by marked loss of body weight, it should, of course, be expected that loss of weight of the ductless glands will occur simultaneously. The point of interest, then, is the final weight of the endocrines expressed as percentage of the body weight. In other words, is the loss in weight of endocrines in avitaminotic animals percentually greater or less than in the litter-mate controls? The increase and decrease in endocrine weights of the pathological animals expressed as percentage of body weight, compared with the controls restricted to the same plane of nutrition, are referred to in this paper as 'hypertrophy' and 'atrophy' respectively.

METHODS

The endocrine weights were studied in the following avitaminoses: Deficiencies of vitamins B_1, B_2 (or G), B_6, vitamin B complex, A, and D. Before an analysis is made of the results submitted, an explanation should be made of the B vitamins. The vitamin-B complex represents a composite of what is known as B_1, the deficiency of which produces polyneuritis with or without convulsions; B_4, deficiency of which results in swollen digits and paws and muscular incoördination; B_2 (or G) which is again a composite of at least two dietary essentials, riboflavin, a deficiency of which results in cataracts; and what is tentatively known as B_6, the deficiency of which produces a dermatitis, frequently referred to as 'pellagra-like symptoms.' Vitamins B_3 and B_5 are dietary factors essential for the pigeon but not for the mammalian rat. Evidence

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is now accumulating which points to the existence of another factor, referred to as the 'maturation factor' (2) a deficiency of which results in a blood disease, similar to aplastic anemia in man, termed 'panmyelophthisis.' This disease is characterized by a pronounced red-cell anemia together with marked reduction in white blood corpuscles and blood platelets. It is associated particularly with extensive hemorrhages. It is produced in the rat on a diet containing as a source of the B vitamins, B_1, B_4, riboflavin, and B_6. It is cured by nicotinic acid. Since nicotinic acid seems to be the human antipellagric factor, (3) it is possible that a deficiency of the 'maturation factor' may be a complicating dietary essential in human pellagra. All these vitamins (with the exception of B_5 which is a maintenance factor required by the pigeon) are growth-promoting dietary essentials.

TABLE 1. INFLUENCE OF AVITAMINOSES ON ENDOCRINE WEIGHTS (SUMMARIZED DATA)

Avitaminosis		Pancreas		Adrenals		Thymus		Thyroids		Pituitary	
		gm.	No. of Groups	gm.	No. of Groups	gm.	No. of Groups	gm.	No. of Groups	gm.	No. of Groups
Vitamin B_1	P	.484	24	.0276	14	.0553	14	.0092	14	.0059	14
	C	.722		.0366		.2697		.0107		.0080	
Vitamin B_2 (G)	P	.530	34	.0331	34	.0808	36	.0118	36	.0067	36
	C	.757		.0350		.2513		.0130		.0085	
Vitamin B_6	P			.0290	21	.1020	21	.0056	21	.0059	21
	C			.0349		.1860		.0064		.0080	
Vitamin-B Complex	P	.610	29	.0308	21	.0776	21	.0114	21	.0066	21
	C	.702		.0378		.1910		.0114		.0080	
Vitamin A	P	.774	60	.0700	14	.1380	14	.0115	14	.0093	14
	C	.817		.0530		.2290		.0123		.0170	
Vitamin D	P	.461	38	.0222	38	.1742	38	.0065	38	.0043	38
	C	.480		.0212		.1608		.0071		.0041	

P = pathological. C = control.

The vitamin-B_1 deficient rations had the following composition: casein, 16; salts (185) 4; Fleischman's yeast, autoclaved for 5 hours at 15 pounds pressure, 7; beef, autoclaved for 6 hours at 20 pounds pressure, 8; filtered butter fat, 9; and cornstarch, 56. The control animals received 20 γ daily of pure vitamin B_1 (thiamin).

The composition of the vitamin-B_2 and A and D deficient rations and of rations deficient in the vitamin-B complex has been outlined in previous publications (1, 4, 5, 6).

In examining the results of B_2 deficiency, a double deficiency of riboflavin and B_6 must be considered. The B_6 deficiency was produced in diets supplemented with 20 γ of pure crystalline vitamin B_1 and 20 γ synthetic riboflavin daily. Since the existence of the 'maturation factor' is of most recent origin, it is possible that the B_6 deficient diets were also deficient in the former component.

In the interpretation of the data, it is significant not only to note the percentual hypertrophy or atrophy of the various endocrines, but also to consider

what percentage of the total groups of animals showed one change or the other. It is much more significant, for instance, that 93 per cent of the groups showed a 50 per cent hypertrophy of the thyroids in vitamin B_1 deficiency than if only 60 per cent had shown it. Insignificant changes will not be included

TABLE 2. INFLUENCE OF AVITAMINOSES ON CHANGES IN ENDOCRINE WEIGHTS OF PATHOLOGICAL ANIMALS COMPARED WITH CONTROLS EXPRESSED ON A PERCENTAGE BASIS (SUMMARIZED DATA)

Avitaminosis	Endocrine	Average for all Groups (% of BW)		% Increase Pathological	% Decrease Pathological	% Showing Increase	% Showing Decrease	% Showing no Change	No. of Groups Studied
		P	C						
Vitamin B_1	Pancreas	.505	.450	12.2	—	79	21	0	24
	Adrenals	.030	.020	50.0	—	93	7	0	14
	Thymus	.059	.140	—	57.1	0	100	0	14
	Thyroid	.010	.006	66.6	—	100	0	0	14
	Pituitary	.007	.005	40.0	—	69	15.5	15.5	14
Vitamin B_2 (G)	Pancreas	.472	.392	20.0	—	85	15	0	34
	Adrenals	.031	.018	71.7	—	88	6	6	36
	Thymus	.068	.134	—	43.2	9	88	3	36
	Thyroid	.010	.007	42.8	—	82	15	3	36
	Pituitary	.005	.004	25.0	—	82	15	3	36
Vitamin B_6	Pancreas	—	—	—	—	—	—	—	—
	Adrenals	.020	.018	11.1	—	66.3	19.6	14.4	21
	Thymus	.068	.096	—	29.2	19.0	81.0	0	21
	Thyroid	.0035	.003	16.6	—	66.6	9.6	23.8	21
	Pituitary	.0035	.004	—	7.0	19.0	38.1	42.9	21
Vitamin-B Complex	Pancreas	.459	.419	9.5	—	82.7	13.8	3.5	29
	Adrenals	.024	.024	±0	±0	55.0	30.0	15.0	20
	Thymus	.062	.120	—	48.3	—	100.0	0	20
	Thyroid	.009	.007	28.3	—	65.0	0	35.0	20
	Pituitary	.005	.005	±0	±0	50.0	30.0	20.0	20
Vitamin A	Pancreas	.582	.416	40.0	—	81.7	10.0	8.3	60
	Adrenals	.026	.020	30.0	—	83.3	16.7	—	12
	Thymus	.099	.128	—	22.6	25.0	75.0	—	12
	Thyroid	.005	.005	±0	±0	50.0	25.0	25.0	12
	Pituitary	.006	.005	16.6	20.0	58.3	25.0	16.7	12
Vitamin D	Pancreas	.598	.650	8.0	—	31.6	68.4	0	38
	Adrenals	.029	.028	3.6	—	42.1	50.0	7.9	38
	Thymus	.226	.216	4.6	—	64.9	35.1	0	38
	Thyroid	.008	.009	—	11.1	43.2	29.8	27.0	38
	Pituitary	.005	.005	±0	±0	30.5	61.1	8.4	38

P = pathological. C = control. BW = body weight.

in the presentation of these data. Space does not permit the inclusion of all our detailed findings; therefore, only summarized data are presented in tables 1, 2, and 3.

RESULTS

Pancreas. A 40-per cent hypertrophy in vitamin-A deficiency in 60 groups, compared with 12.2 per cent in vitamin-B_1 deficiency in 24 groups, is significant. It is also interesting to note that 81.7 per cent of the pathological animals in the A avitaminotic state showed that change in the pancreatic weight.

Adrenals and thyroids. That there is adrenal hypertrophy in vitamin B_1 deficiency has been known for years, and it was thought that this was the only endocrine whose weight actually becomes greater in proportion to body weight during B_1 avitaminosis. It is now apparent from the data presented in 14 groups of animals that hypertrophy of the thyroids is even more striking in this gland than in the adrenals, in this avitaminosis. The adrenal hypertrophy is still more marked in vitamin B_2 than in vitamin B_1 deficiency. Although 55 per cent of the groups show increase in adrenal weights in a deficiency of the vitamin-B complex, the 30 per cent that showed decrease were so much greater that, when averages are taken for all animals, no change is evident as compared with the controls. Since animals on diets deficient in the vitamin-B complex do not live long enough for definite symptomatology of avitaminotic states to

TABLE 3. INFLUENCE OF REPEATED VITAMIN-B_1 DEPLETIONS ON THE WEIGHTS OF ENDOCRINES

Endocrine	Average for all groups (% of BW)		% Increase Pathological	% Decrease Pathological	% Groups Showing Increase	% Groups Showing Decrease	% Groups Showing no Change	No. of Groups Studied
	P	C						
Adrenals								
S.D.	.030	.020	50.0	—	93	7	0	14
R.D.	.024	.020	20.0	—	72	28	0	13
Thymus								
S.D.	.059	.140	—	57.1	0	100	0	14
R.D.	.037	.056	—	34.0	15	85	0	13
Thyroid								
S.D.	.010	.006	66.6	—	100	0	0	14
R.D.	.0060	.0055	9.1	—	25	33	42.0	12
Pituitary								
S.D.	.007	.005	40.0	—	69	15.5	15.5	14
R.D.	.005	.005	±0	±0	±0	±0	±0	10

S.D. = single depletion. P = pathological.
R.D. = repeated depletions. C = control.

develop, the loss of weights of the adrenals in a large number of animals seems to go on parallel or even more rapidly than does loss of body weight; hence, the lack of adrenal hypertrophy. The thyroid hypertrophy is thus also considerably diminished. It is interesting to note that there is also hypertrophy of the adrenals in vitamin-A deficiency but only three-fifths as great as in vitamin-B_1 deficiency.

Thymus. Perhaps the most noteworthy change in endocrine weights in avitaminoses is the marked atrophy of the thymus in B_1 deficiency which occurred in 100 per cent of the pathological animals. As already stated, the atrophy was correlated with histological observations. The atrophy of the thymus is also very pronounced in human beriberi. Atrophy of the thymus is also marked in vitamin B_2 deficiency and in a deficiency of the vitamin-B complex. In great contrast to this, the degree of thymic atrophy in vitamin-A deficiency is relatively mild, being only 28.6 per cent as found in 9 groups out of 12.

Pituitary. The only avitaminoses in which hypertrophy of the pituitary was observed are B_1 and B_2. The extent of hypertrophy was 40 and 25 per cent respectively.

Endocrine weights in vitamin-D deficiency. No noteworthy changes in endocrine weights were observed in this avitaminosis, probably because there has not been sufficient time for changes to develop, since the experimental period lasted only 35 to 40 days.

Influence of Repeated Vitamin-B_1 Depletions on the Weights of Endocrines

Hitherto biochemical and histopathological changes in avitaminoses were studied in animals that were allowed to exhaust their vitamin reserves during varying stages of a single depletion period. It was suggested by Dr. Lincoln Opper, the histopathologist of this department, that after the first depletion

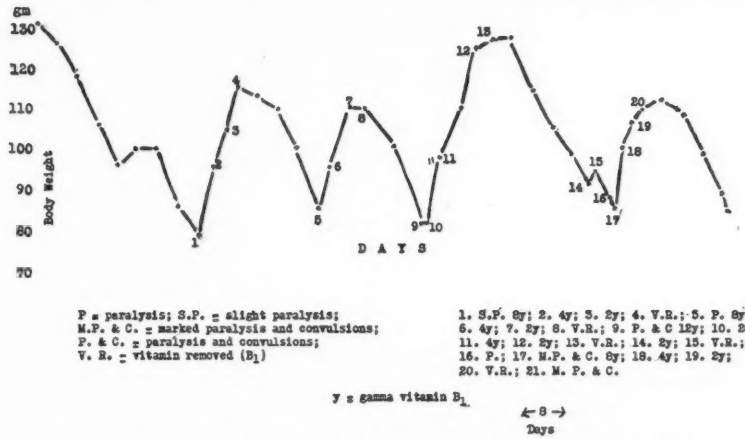
gm
130
120
110
100
90
80
70
Body Weight
DAYS
P = paralysis; S.P. = slight paralysis;
M.P. & C. = marked paralysis and convulsions;
P. & C. = paralysis and convulsions;
V. R. = vitamin removed (B_1)
1. S.P. 8y; 2. 4y; 3. 2y; 4. V.R.; 5. P. 8y;
6. 4y; 7. 2y; 8. V.R.; 9. P. & C 12y; 10. 20y;
11. 4y; 12. 2y; 13. V.R.; 14. 2y; 15. V.R.;
16. P.; 17. M.P. & C. 8y; 18. 4y; 19. 2y;
20. V.R.; 21. M. P. & C.
y = gamma vitamin B_1
8
Days

Fig. 1. (Exp. 3830) Influence of repeated vitamin B_1 depletions and therapy on growth and polyneuritis.

is reached in various vitamin-deficiency diseases the animal be given vitamin therapy and then allowed to deplete again, and if this operation were repeated several times, we might be able to produce a chronic state of avitaminosis, and additional pathological states in the tissues might be encountered. By this technic we have kept several dozen groups of vitamin-B_1 deficient animals alive and in a chronic state of avitaminotic flux for 6 to 10 months, some animals having undergone as many as 8 depletion periods. During the moribund stage of marked paralysis and convulsions we always succeeded, with one exception, in saving the animals with 10 γ crystalline B_1. In figure 1 a typical case of repeated vitamin-B_1 depletion is presented graphically.

In table 3 the results of repeated vitamin-B_1 depletions in 10 to 13 groups of animals are compared with the results of single depletions in 14 groups of animals. It will be noted that, although during a single vitamin-B_1 depletion period, the loss of weight of the adrenals, thyroid, and pituitary is relatively far less than that of the whole body (in other words, there is a relative hypertrophy of these endocrines), in repeated vitamin-B_1 depletions

the hypertrophy in the pituitary entirely disappears; in the thyroid it is reduced to insignificant proportions (9.1 per cent); and in the adrenals the hypertrophy is reduced from 50 to 20 per cent. All of these figures are relative to fluctuations in the body weight. In other words, it takes repeated bombardments of the animal to several severe depletions before the extent of loss of weight of these endocrines begins to approximate that of the body as a whole.

It will also be noted that the influence of repeated vitamin-B_1 depletions produced a less marked atrophy in thymus than had been produced by a single depletion.

With the exception of atrophy of the thymus in beriberi, no information is available on endocrine weights in various states of avitaminoses in the human. No correlation, therefore, can be made at present between the results submitted in this communication and human experiences.

SUMMARY

The influence of various avitaminoses on weights of endocrines has been investigated. The most significant observations follow. In vitamin-A deficiency in 60 groups of animals there was observed an hypertrophy of the pancreas, amounting to 40 per cent. In vitamin-B_1 deficiency there is a marked hypertrophy of the thyroids and adrenals. The most noteworthy change in endocrine weights in avitaminoses is the marked atrophy of the thymus in vitamin-B_1 deficiency which occurred in 100 per cent of the pathological animals. The atrophy was correlated with histological observations. Hypertrophy of the pituitary was found in vitamin-B_1 and B_2 deficiency to the extent of 40 and 25 per cent respectively.

Repeated vitamin-B_1 depletions on endocrine weights have the following influence: The hypertrophy of pituitary entirely disappears; in the thyroid it is reduced to insignificant proportions (9.1 per cent) and in the adrenals it is reduced from 50 to 20 per cent.

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A MODIFIED AND IMPROVED METHOD FOR THE PREPARATION OF THYMUS EXTRACT

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In previous publications (1, 2) it was stated that all biologically potent preparations of thymus extract contained iodine-reducing material in excess of 15 mg. per cent. With this as an arbitrary criterion, the original method as published by Hanson (3) was modified so that conditions would be more favorable for increasing this iodine-reducing titre (i.r.t). Over a period of several years, additional improvements were made until, at present, values averaging 250 mg. per cent are regularly obtained.

Whether this iodine-reducing material is associated with the active principle is not known; however, it is believed that there is a positive correlation between the high i.r.t. and the rapidity and efficiency of collection, and extraction. This is substantiated by the fact that extracts which were initially high in i.r.t. were found active after several months when the i.r.t. was very low. Glands, which had been allowed to remain at room temperature after the calf had been killed and thereby had begun to autolyze, had a much lower i.r.t.

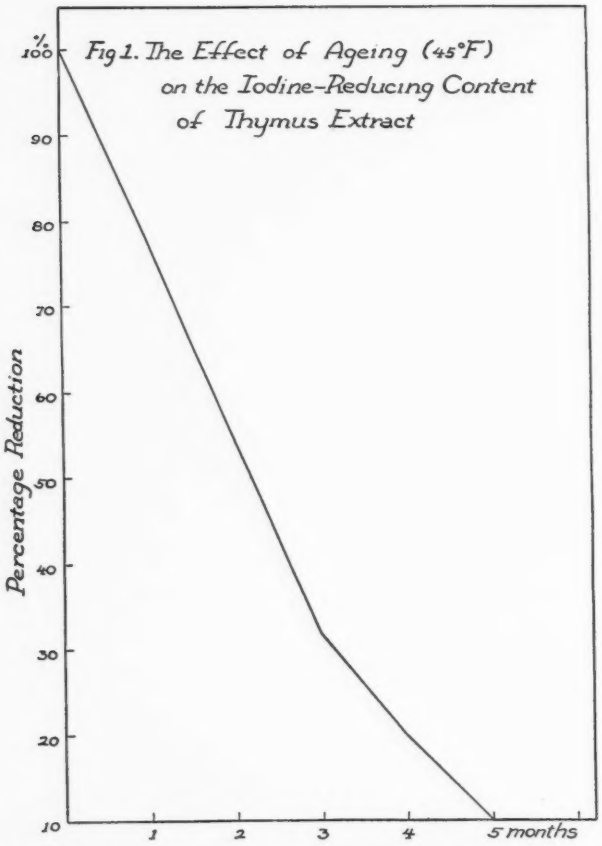
TABLE 1. THE SEASONAL VALUES OF THE I.R.T. IN THYMUS EXTRACTS

Extract	Month	i.r.t. mg. %	Tissue per 1 cc. gm.	Extract	Month	i.r.t. mg. %	Tissue per 1 cc. gm.
1	Jan.	130	1.0	14	Aug.	112	1.0
2	Jan.	200	1.0	15	Sept.	186	1.0
3	Jan.	190	1.0	16	Sept.	169	1.0
4	Feb.	200	1.0	17	Sept.	239	1.5
5	Mar.	215	1.0	18	Oct.	371	1.5
6	Apr.	240	1.0	19	Oct.	290	1.5
7	Apr.	200	1.0	20	Nov.	359	1.5
8	May	240	1.0	21	Nov.	359	1.5
9	June	160	1.0	22	Nov.	370	1.5
10	July	110	1.0	23	Dec.	275	1.5
11	July	90	1.0	24	Dec.	375	1.5
12	Aug.	120	1.0	25	Jan.	210	1.5
13	Aug.	100	1.0	26	Jan.	225	1.5
				27	Jan.	226	1.5

It has been possible, by means of these changes, to decrease also the toxicity of the extract. Five cc. of the original extract was sufficient to cause death in a 100 gm. rat, due to auriculo-ventricular heart block, while as much as 18 cc. of the present extract may be administered under similar conditions with safety. With the modified extract it has also been possible to effect precocity in growth and development in the second instead of the third generation.

EXPERIMENTAL PROCEDURE

The technic for the preparation of the extract is given as follows. The thymus glands of locally raised[1] 2 to 6-week-old calves are obtained fresh and immediately ground into a measured amount of 1 per cent HCl (by volume). The container is placed in a pail of brine and transported to the laboratory, where an additional amount of the same acid is added so that 1500 gm. of



fresh, raw thymus tissue is extracted with 1000 cc. of 1 per cent HCl. (Attempts to use more than 1.5 gm. of tissue per 1 cc. of acid have resulted in increased toxicity and protein in the final product.) The mixture is then heated directly and rapidly, with continuous stirring, to 68°C., when the tissue coagulates and sediments, leaving a clear, pale yellow supernatent fluid. The latter may be removed either by filtration through coarse filter paper, syphoning or centrifugation, and 0.2 per cent chlorbutanol is added as a preservative.

[1] It has been found that these glands are in much better condition and yield an extract of a higher i.r.t. than those of calves that have been shipped from long distances.

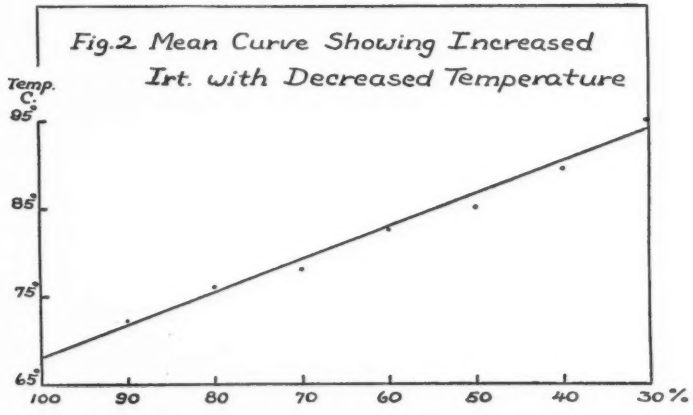
TABLE 2. A COMPARISON IN THE NITROGEN VALUES

	Tot. N	N.P.N.	Tot. Protein
	percentage		
Original Extract	5.75	1.55	4.20
Modified Extract	0.43	0.42	0.008

By heating the extract a little less rapidly, it is possible to demonstrate the coagulation point at 55-60°C., but this extract will contain a small amount of protein, whereas that obtained with the outlined procedure is practically protein-free (table 2). The total solids in this extract is 2.15 gm. per 100 cc.

The extract is stored in bulk in pyrex flasks at a temperature of 40°F., and is used as such for animal experimentation or passed through a Berkfeld candle and bottled in 10 cc. hard glass vials for human use.

Table 1 illustrates the i.r.t. of a series of extracts prepared by this method, and indicates an apparent seasonal variation in the i.r.t. which seems to be much higher during the colder months than in the summer time. Figure 1 illustrates the rate of deterioration in the i.r.t. on ageing, and indicates that the i.r.t. decreases by about 20 per cent each month until, at the end of 5 months, it is not demonstrable. Figure 2 shows the increased i.r.t. obtained with decreased temperatures.



The final test in determining the biological potency of the extract is based on the demonstration of precocious growth and development in the offspring of an advanced generation female rat treated solely with the extract to be assayed.

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CERTAIN IODINE-REDUCING SUBSTANCES OF THYMUS EXTRACT[1]

BIOLOGICAL CONSIDERATIONS

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In previous reports it has been shown that the administration of thymus extract (1) or homologous thymus implants (2, 3) through successive generations of rats resulted in accruing acceleration in the rate of growth and development of the offspring. It has been demonstrated that thymectomy (4, 5, 6), in successive generations of rats has resulted in retardation, which may be corrected by either the administration of thymus extract (4, 5, 7) or homologous thymus implants (4, 8). It has also been found (9) that all biologically potent extracts of thymus contained appreciable amounts of iodine-reducing material (50 to 258 mg. per cent, calculated as glutathione) by the method of Woodward and Fry (10) and that this was low, or absent, in all biologically inactive preparations. In this report is demonstrated the biological effects of certain iodine-reducing substances associated with iodine-reducing material in the thymus extract, as well as a comparison between the effects of these substances and those obtained with thymus extract itself.

Suggestive evidence of the biological activity of glutathione and cysteine has been previously published (9, 11). Without assuming a necessary relationship between the iodine-reducing content and the potency of the extract, several known naturally occurring iodine-reducing compounds, namely glutathione, cysteine and ascorbic acid, were arbitrarily selected and biologically investigated. It was this relationship which furnished the initial lead into the accompanying chemical analysis (12) of the iodine-reducing fraction of the thymus extract.

The thymus extract administered in these experiments and utilized for chemical analysis was a modification by Steinberg (13) of the original extract prepared by Hanson (14).

In our earlier experiments based on the injection of glutathione, and cysteine in successive generations, it was noted that the effects were meager and often inconstant. This was later thought to be due to the disintegration of the products used, which were made up in stock solutions, and used for a week when a fresh supply was prepared. Careful examination of these former results seemed to indicate that precocity was apparent only when the solutions were

[1] This work was aided by grants from the Penrose Fund of the American Philosophical Society, and the Rockefeller Foundation.

reasonably fresh. Therefore, in the present studies, daily intraperitoneal injections of freshly prepared solutions of these iodine-reducing compounds (made by dissolving a weighed amount of substance in dilute hydrochloric acid at pH of 4.6) have been used. In this way deterioration is appreciably diminished.

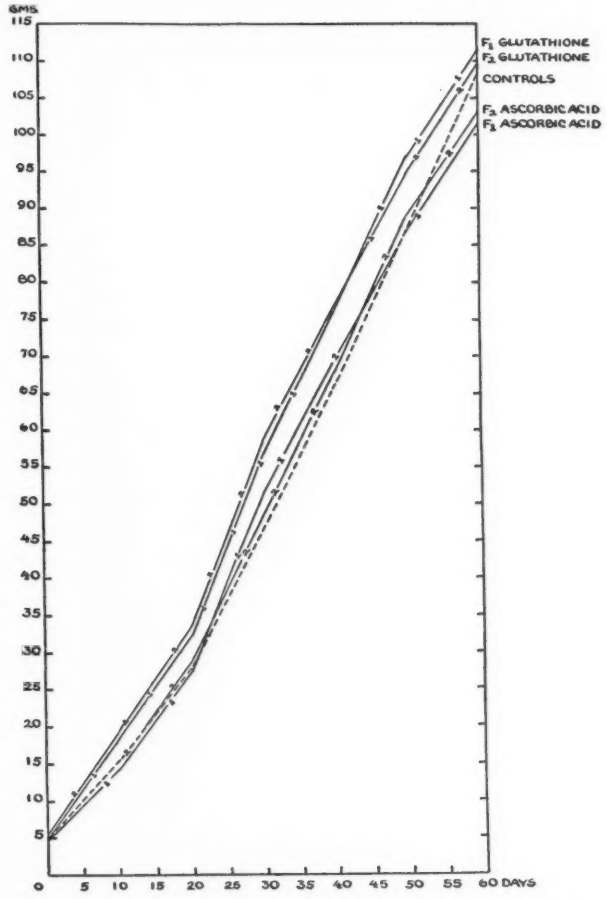

Fig. 1. Growth curves of successive generations of glutathione, and ascorbic acid test rats contrasted with controls.

Recent experiments have shown that it was not necessary to inject both parents with thymus extract in order to produce the increased growth and development observed in the offspring.[2] Experiments were simultaneously conducted in which the male parent alone was treated, the female parent alone, and both parents were treated. The results of these experiments indicated that

[2] Experimental data will be presented at an early date in a separate communication.

the injection of the male alone was not effective, however, when the female alone or both parents were injected, the development of the offspring was similar. Corresponding results with the treatment of the female separately and both parents together were also obtained in the present series of experiments utilizing glutathione, ascorbic acid, cysteine and the combination of glutathione and ascorbic acid.

Effects of administration of glutathione. In order to determine if glutathione[3] was related to the iodine-reducing material of the thymus extract, the former was investigated independently by two workers in this laboratory (A. S. and N. H. E.). It was found that no definite or constant effects resulted from 0.25 mg. daily (106 rats) or 1 mg. daily (87 rats), but when a 200 mg. per cent solution was administered daily to successive generations of rats in 1 cc. dosage, the rate of growth was increased by 20 per cent during the first 40

TABLE 1. SOMATIC DEVELOPMENT OF GLUTATHIONE-TREATED TEST RATS

	(A.S.)	(N.H.E.)	Total average	(A.S.)	(N.H.E.)	Total average	Controls[1]
Generation	F_1		F_1	F_2		F_2	
Dosage, mg.	2		2	2		2	
No. of rats	90	113	203	42	87	129	
Birth weight, gm.	4.9	4.9	4.9	5.3	5	5.2	4.9
Ears detached, days,	1.7	2.1	1.9	1.6	1.9	1.7	2½–3½
Teeth erupted, days,	6.0	6.9	6.5	6.5	6.4	6.4	8–10
Hair appeared, days,	11.0	11.2	11.1	11.0	10.9	10.9	12–16
Eyes opened, days,	11.3	12.3	11.8	10.3	10.6	10.5	14–17
Testes descended, days,	17.0	22.3	19.9	15.0	21.0	20.6	35–40
Vagina opened, days,	28.0	39	34.1	29.0	37.8	34.9	55–62

[1] Data on controls are based on 400 animals, observed over a period of 3 years. These values have been used in all experiments reported in this communication.

days after birth, thereafter approaching normal at about 60 days in the second generation, F_1 (fig. 1). In addition, early somatic differentiation was also observed (table 1); viz., the teeth erupted at an average of 6.5 days instead of 9 days, while the eyes opened at 11.8 days instead of 15.2 days. Gonadal development was also markedly accelerated.

In the third generation (F_2), the accruing effect was not as marked as in the third generation in the thymus-injected rats, viz., the rate of growth was increased about 25 per cent, and the teeth erupted at 6.4 days. However, the eyes opened at 10.5 days, which was somewhat earlier than the F_1 group. Gonadal development in the F_2 was about 50 per cent accelerated over that of the controls. The mortality was about 25 per cent.

Effects of administration of ascorbic acid.[4] The possibility of ascorbic acid being similarly associated with the iodine-reducing material was also considered. No precocity in growth or development was observed in 95 rats in the second generation with 0.25 mg. daily, nor in 78 rats with 1 mg. daily. It was necessary to inject 1 cc. of a 200 mg. per cent solution intraperitoneally daily (table 2) before biological effects were obtained. No significant increase in

[3] The glutathione used was both commercial (Pfanstiehl) and that prepared in the laboratory by Pirie's method (14) with an additional copper precipitation.

[4] Cebione, generously supplied by Merck and Company, was the sole source of ascorbic acid utilized in these experiments.

bodily growth was observed in the second generation. However, somatic development was for the most part slightly better than that observed in the glutathione group. Analysis of table 2 revealed that in the second generation (F_1) the teeth erupted at 6.1 and 5.6 days, or an average of 5.8 days, while the eyes opened at 11.3 and 10.3 days, or an average of 10.7 days. Here again,

TABLE 2. SOMATIC DEVELOPMENT OF ASCORBIC ACID-TREATED TEST RATS

	(A.S.)	(N.H.E.)	Total average	(A.S.)	(N.H.E.)	Total average
Generation	F_1		F_1	F_2		F_2
Dosage, mg.	2		2	2		2
No. of rats	91	88	179	34	51	85
Birth weight, gm.	4.9	4.8	4.9	5.1	4.8	4.9
Ears detached, days,	1.9	2.2	2.0	1.8	2.1	1.9
Teeth erupted, days,	5.6	6.1	5.8	6.0	6.0	6.0
Hair appeared, days,	10.0	11.0	10.4	10.0	10.2	10.1
Eyes opened, days,	10.3	11.3	10.7	9.4	11.0	10.3
Testes descended, days,	13.0	22.4	17.6	13.2	20.8	17.7
Vagina opened, days,	25.0	40.1	32.4	23.0	36.7	31.2

gonadal development seemed to be about 50 per cent accelerated over that of the controls.

In the third generation (F_2), there was also no significant increase in weight. The acceleration in somatic development seemed to be more marked than any of the previous groups, for here the teeth erupted at an average of 6 days, while the eyes opened at 11 and 9.4 days, with an average of 10.3 days. Gonadal development was increased slightly more than 50 per cent over that of the controls.

The mortality in the ascorbic acid group was higher than that observed in the glutathione group, being about 35 per cent.

Effects of administration of cysteine. Cysteine[5] in the form of the hydro-

TABLE 3. SOMATIC DEVELOPMENT OF CYSTEINE-TREATED TEST RATS

	(A.S.)	(N.H.E.)	Total average	(A.S.)	(N.H.E.)	Total average
Generation	F_1		F_1	F_2		F_2
Dosage, mg.	1		1	1		1
No. of rats	54	71	125	19	36	55
Birth weight, gm.	4.8	4.9	4.9	4.2	4.8	4.6
Ears detached, days,	2.0	2.1	2.1	1.5	2.1	1.9
Teeth erupted, days,	6.0	6.2	6.1	5.0	5.1	5.7
Hair appeared, days,	9.0	11.2	10.2	9.0	11.3	10.5
Eyes opened, days,	10.1	11.8	11.1	9.5	11.5	10.8
Testes descended, days,	14.1	22.9	19.1	16.0	20.3	18.8
Vagina opened, days,	30.1	40.1	35.7	30.0	38.6	35.6

chloride was likewise administered in the dosage of 0.25 mg. and 1 mg. per rat per day. This substance was selected because it is the iodine-reducing constituent of glutathione. No results were seen in 97 rats born to parents getting 0.25 mg. per day. Much difficulty was experienced in its utilization in earlier experiments. Moreover, in the present work, cysteine has been found

[5] The cysteine hydrochloride used was commercial (Pfanstiehl).

to be quite toxic when given daily in the dosage used. It is for this reason that so few animals (table 3) survived sufficiently long to permit of prolonged observation.

With 1 mg. of cysteine per rat per day (125 rats) the rate of growth of the test rats of the second generation (F_1) was essentially normal with a tendency to fall below normal after the offspring themselves were placed on treatment. It is not unlikely that this slowing in growth was due to the toxic effects of the cysteine itself, for the animals evidence irritation from the injection. However, in spite of these untoward effects, the rate of somatic differentiation was appreciably increased as evidenced by the teeth erupting at 6.1 days instead of at 9 days, and the eyes opening at 11.1 days instead of 15.2 days.

TABLE 4. SOMATIC DEVELOPMENT OF GLUTATHIONE PLUS ASCORBIC ACID-TREATED TEST RATS

Generation	F_1	
Dosage	1 mg. GSH+1 mg. AA	
No. of rats	36	
Birth weight, gm.	4.8	
	Range	Average
Ears detached, days,	1– 3	2.0
Teeth erupted, days,	6– 7	6.2
Hair appeared, days,	10–13	11.0
Eyes opened, days,	8–11	9.6
Testes descended, days,	14–20	16.0
Vagina opened, days,	16–30	23.0

In the 55 third generation (F_2) rats, despite the birth weight being lower than the controls (table 3) the rate of somatic development was significantly greater than in the previous generation (F_1). The teeth erupted at an average of 5.7 days, the eyes opened as early as 9.7 days with an average of 10.8 days, and the gonadal development was slightly more accelerated.

The mortality in this group was one of the highest of all these experimental groups, being at least 50 per cent.

The effects of the simultaneous administration of glutathione and ascorbic acid. In view of the increase in growth in the offspring of glutathione treated animals, and the acceleration in development in the offspring of ascorbic acid treated animals, it was thought advisable to inject a combination of ascorbic acid and glutathione, 1 mg. each, with the possibility of securing a more marked effect. Although there have been to date only 36 animals in the second generation (F_1), it appears that in these the rate of growth is about equivalent to that obtained with glutathione alone (table 4). Somatic development was also markedly precocious; the teeth erupted at 6.2 days or an increase of 31 per cent over normal, and the eyes opened at 9.6 days or an increase of 36.8 per cent above normal. Gonadal development was increased more than 50 per cent. The animals of the F_1 generation have not bred until recently, and F_2 data is insufficient for this report. No biological results have been obtained with 0.5 mg. glutathione, and 0.5 mg. ascorbic acid in combination.

Comparison of thymus effects, F_1 and F_2, with glutathione effect, F_1 and F_2. A comparison of the third generation (F_2) glutathione and thymus-treated rats (table 5) reveals a similarity in birth weight, detachment of ears, and eruption of incisors. However, both the appearance of hair and the opening of eyes is accelerated by approximately 50 per cent in the thymus-treated animals; while gonadal development is more precocious in the glutathione treated rats. In the analysis of the weight chart (table 5) in the F_1 generation, the glutathione-treated rats grew more rapidly than the thymus-treated rats for the first 50 days and thereafter approaching normal at about 60 to 70 days, whereas the growth curve of the thymus-treated rats did not coincide with that of the controls until about 120 days. In the F_2 generation, the thymus-treated rats manifested a much greater increase in the rate of growth from birth, and at 60 days were 13 per cent heavier than the glutathione treated animals. From

TABLE 5. COMPARISON OF BODY WEIGHTS AT 10-DAY INTERVALS OF GLUTATHIONE AND THYMUS-TREATED TEST RATS

Generation	F_1	F_1	F_2	F_2	Controls
Series	Thymus	GSH[1]	Thymus	GSH	
Birth	5.1	4.9	5.3	5.4	4.9
10 days	20.0	18.3	27.0	19.2	14.0
20 days	36.0	32.3	52.0	33.6	27.0
30 days	47.0	56.5	78.0	58.5	47.0
40 days	66.0	75.8	105.0	76.5	65.0
50 days	87.0	96.6	128.0	94.8	88.0
60 days	138.0	111.3	148.0	109.3	108.0

[1] Data on controls are based on 400 animals observed over a period of 3 years. These values have been used in all experiments reported in this communication.

this comparison between the biological effects of thymus extract, glutathione, ascorbic acid, cysteine, and a combination of glutathione and ascorbic acid, it seems that the latter substances, when injected under similar conditions, do not account for all the biological activity of thymus extract.

DISCUSSION

When the original results on the injection of thymus extract in successive generations of parent rats were published, both parents were treated. Positive results have since been obtained when the female alone was treated. This is found to apply to all of the substances here used, i.e., precocious growth and development (one or both) have been observed in the offspring when only the mothers have undergone injection with glutathione, ascorbic acid, and cysteine. In thymectomized animals it was found necessary to ablate the gland of both parents in order to demonstrate retardation in the offspring (6). It is interesting to note that thymectomy (16) in the rat has been recently found to lower the glutathione of the liver.

Glutathione, ascorbic acid, and cysteine, separately and in combination, have exhibited the ability to accelerate the rate of growth or development of the offspring (one or both) when injected intraperitoneally in suitable amounts

through successive generations of rats. These results are not obtained, however, with any of these substances, alone or in combination, in amounts up to 1.00 mg. per rat. Although the average fresh extract on analysis contains only 70 mg. per cent glutathione, 30 mg. per cent ascorbic acid, and 5 mg. per cent cysteine (12). The biological effects of these substances are not identical; all three iodine-reducing agents accelerate the rate of development, but only glutathione stimulates the rate of growth in the dosage employed. Furthermore, this effect of the iodine-reducing substances although similar is not as marked, i.e., the accruing nature of the acceleration in growth and development so strikingly exhibited in the thymus-treated group of rats is not as evident in the studies with these iodine-reducing agents. In this connection more evidence is being sought, especially to determine any alteration in the biologic results, with larger doses of these substances.

The retardation in growth and development incident to thymectomy through successive generations of rats is readily overcome by thymus extract, but recently it has been found that it is entirely unaffected by glutathione, ascorbic acid and cysteine in the amount used to date.[6]

Regardless of the rôle that these substances may play in the activity of the thymus extract, their own biological properties are interesting in themselves. Since there is evidence that certain sulfhydryl compounds (17, 18, 19) and ascorbic acid (20) stimulate cell proliferation, they may play the same rôle here; however, this appears to be precluded by the lack of stimulation in thymectomized rats.

SUMMARY

Glutathione, ascorbic acid and cysteine are all capable in the dosage herein employed, of accelerating the rate of development of the offspring of treated rats, whereas glutathione in addition is capable of accelerating growth. The biologic effects of these iodine-reducing substances simulate those of thymus extract in many respects. The iodine-reducing substances do not appear to be wholly responsible for the biologic effects, as evidenced by the following. *a)*, They fail to approximate the thymus effects quantitatively; *b)*, They do not yield marked accruing effects as evidenced through successive generations, and *c)*, they fail to overcome the retardation incident to thymectomy which, on the other hand, is readily controlled by thymus extract, and *d)*, extracts were found to be active after several months when the iodine-reducing value was very low (13).

We are indebted to the late Dr. H. H. Donaldson of the Wistar Institute for his kindly interest, unfaltering faith, and invaluable counsel.

We also acknowledge our indebtedness for technical assistance to M. D. McCarthy and Miss Louise Tinnermann.

APPENDIX

In the discussion of this paper at the Association meeting in Atlantic City, June 8, 1937, additional confirmatory evidence was presented by Dr.

[6] Unpublished work of Einhorn and Rowntree.

Albert C. Buckley, from the Friends Hospital, Philadelphia, Pa. and by Dr. Milton Lee, from the Harvard Medical School, Boston, Mass. An abstract of this part of the discussion follows.

Dr. Albert C. Buckley: About two years ago, when I first had the opportunity to observe the results of Dr. Rowntree's work in the production of precocity in albino rats, in successive generations, by the injection of extract of thymus gland, it occurred to me that a study of the developmental progress of the central nervous system would be of interest.

It may be stated that one of the important indications of an advance in development is the appearance of myelin substance ensheathing the axis cylinders of nerve fibers in the several pathways, which takes place in a definite order.

It has already been established by Donaldson (1901), and by Watson (1903), that there are no myelinated fibers in the central nervous system of the albino rat normally at birth or at 24 hours after birth. At 48 hours a few scattered fibers occur myelinated in the anterior and lateral columns of the spinal cord. The process of myelination progresses each day thereafter and at the sixth day myelinated fibers are seen for the first time in the anterior commisure.

Because of the well defined myelination at this age the 6-day animal was selected for comparison with treated animal in our controls, and those established by Watson.

The spinal cords of the sixth-day treated animal showed myelination equivalent to the 13-day normal control, in the 6th generation. In the 10th generation of treated animals myelination is comparable with the 20-day normal controls.

At 48 and 24 hours, in treated animals myelinated fibers are demonstrable in all pathways. Furthermore, at birth myelination has been observed. To completely eliminate any doubt as to the age of the animal the new-born used for study was personally observed by me in the process of birth.

Dr. Milton Lee: In the earlier reports by Rowntree and his collaborators (J.A.M.A. **103:** 1425; Science **83:** 443) it was apparent that the method of preparing the thymus extract used was such that glutathione and similar reducing substances would be extracted and preserved. Gregory and Goss have shown that the embryos and newborn animals of certain varieties of large rabbits have a higher GSH concentration in their tissues than embryos and new-born of varieties of small rabbits. Accordingly, in collaboration with Dr. G. B. Ayres, we made some experiments in which glutathione extracted from yeast was injected into successive generations of rats (Wistar strain) after Rowntree's method. The dosage varied from 0.5 mg. (in young) to 1.0 mg. (in adults) per rat per day. Treatment began on the second day after birth in each generation and continued until the females of that generation had borne and successfully weaned a litter. The experiment was carried only through 4 generations.

In the third generation there was some evidence of precocious development and in the fourth generation precocity was unmistakable. In the 26 rats of the fourth generation (4 litters) the average birth weight was 6.5 gm. The ears opened within 36 hours; the teeth erupted within 3 to 4 days; hair appeared within 4 to 7 days; and the eyes opened within 6 to 7 days. In the males the testes descended by the 15th to 19th day; in the females the vagina opened about the 28th day. The average body weight of the females at 25 days was 48 gm. This represents considerably greater precocity than we had ever observed in our colony of rats.

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CERTAIN IODINE-REDUCING SUBSTANCES OF THYMUS EXTRACT

CHEMICAL ANALYSIS OF EXTRACT[1]

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In the preceding paper (1) it was shown that when glutathione, ascorbic acid, and cysteine were administered to rats through successive generations, they accelerated growth and development in the offspring in a similar but not identical way as did the thymus extract. The presence of these and other iodine-reducing substances was naturally sought in the thymus extract.

Both ascorbic acid (2, 3, 4, 5) and glutathione (6) have been previously reported in the thymus gland, the former with the indophenol titration and the latter with the non-specific colorimetric nitroprusside method. The presence of glutathione in the thymus gland has been definitely established by us, by chemical isolation and identification in work which will be reported elsewhere.

In the present paper we report an analysis of the iodine-reducing material of the thymus extract.

METHODS

Ascorbic acid. This was determined by titration with 2-6 dichlorophenol indophenol (7) in the presence of 2 per cent metaphosphoric acid (8) in a volume of 50 cc.

Cysteine. The Sullivan colorimetric method (9) was used. Ascorbic acid was added to the standard in an amount equal to that in the unknown because it inhibited the color (10). Glutathione has no inhibiting action (11).

The acidity of the unknown was adjusted to 0.1 normal, and 2 cc. of 5 per cent sodium cyanide was added. The unknowns were centrifuged. The variations of the method with the extract was at times as great as 10 to 15 per cent. In one determination a Wratten filter No. 58 was employed (12). The cysteine was standardized iodometrically (13).

Glutathione. The Binet and Weller cadmium lactate precipitation method (14) was employed in a modified form. Cadmium lactate precipitates both glutathione and cysteine, but not ascorbic acid and ergothioneine. Cysteine was not initially separated as Binet and Weller suggested, but was quantitatively precipitated along with the glutathione and the iodine reduction of the cysteine, equivalent to that found by the Sullivan determination on the extract, was subtracted from the iodine reduction of the cadmium precipitate to obtain the

[1] This work was aided by grants from the Penrose Fund of the American Philosophical Society. These chemical studies have been made possible through the generosity of the Rockefeller Foundation.

iodine reduction of the glutathione. We have not been able to determine the cysteine in the cadmium precipitate with the Sullivan method either in the presence of the cadmium or after its removal by hydrogen sulfide.

Metaphosphoric acid was added to the extract to bring about a quantitative precipitation of the glutathione which was otherwise less than 100 per cent precipitated under various conditions investigated. Metaphosphoric acid also served as a helpful buffer in the adjustment of the pH when working with standard glutathione and cysteine. The iodine reduction of the cadmium precipitate and cysteine was determined by the method used for the total iodine reduction described below. The cadmium metaphosphate had no effect on the titration. The variation of the cadmium precipitation was about 3 per cent. The glutathione was standardized iodometrically (15).

At least 3 cc. of extract (more with older extract) was added to a 50 cc. conical centrifuge tube, 2 cc. of 2 per cent metaphosphoric acid added, and dilution to 10 cc. was made. 2 cc. of saturated cadmium lactate was added and the pH was brought to 6.8 to 6.9 by the addition of sodium hydroxide, first with 2.5 N and finally with 0.5 and 0.1 N, using the spot plate technic. It was allowed to stand at least one-half hour (or over night) in the refrigerator. It was centrifuged, drained and wiped dry with filter paper. The precipitate was washed twice with 3 cc. of water and titrated after dilution to 2 cc.

Preliminary experiments indicate that metaphosphoric acid may be replaced by ortho-phosphoric acid (1.33 cc. of 0.25 M) and cadmium lactate by 2 cc. of 8.0 per cent $CdCl_2 \cdot 2H_2O$ or 9.8 per cent $Cd(CH_3COO)_2 \cdot 2H_2O$. However, these reagents require further control experiments.

Ergothioneine. The qualitative Hunter reaction (16) was used.

Total iodine reduction. To compare the sum of the iodine reduction of the components with the total iodine reduction, it was obviously important that the latter be additive for the components. This requirement was met in the present titration, i.e., within its accuracy. All the determinations were run as closely together as possible. Empirical iodine-reducing values (table 1) have been

TABLE 1. EMPIRICAL AND THEORETICAL IODATE-TITRATION VALUES FOR IODINE-REDUCING SUBSTANCES. KIO_3=0.004526 N

	Empirical Value cc. per mg.	Theoretical Value cc. per mg.
Glutathione	0.71	0.72
Ascorbic Acid	2.33	2.51
Cysteine	1.82	1.83

approximately established for the concentrations of ascorbic acid, glutathione, and cysteine in the unknowns under the conditions of the titration. An endpoint corresponding to 0.02 cc. of 0.005 N potassium iodate is taken which limits the reproducibility of the average titration to within 2 per cent.

One cc. of boiled-out concentrated hydrochloric acid and 2 cc. of 4 per cent potassium iodide are added to 2 cc. of extract or to the cadmium precipitate, the ascorbic acid, glutathione and cysteine standards in a volume of 2 cc.

It is titrated with with 0.005 N potassium iodate to the yellow end-point (the greatest change which persists) without starch.

RESULTS

With the above methods ascorbic acid, glutathione and cysteine have been shown to be present in the thymus extract and ergothioneine to be absent. In table 2 (*a* and *b*) are given the concentrations and percentages of total iodine reduction of these constituents in 6 thymus extracts varying from 1 to 20 days after preparation. In extracts less than two weeks old, ascorbic acid comprises

TABLE 2. CONCENTRATIONS AND PERCENTAGES OF IODINE-REDUCING CONSTITUENTS OF THYMUS EXTRACT

Extract	Age, days	Ascorbic Acid		Glutathione		Cysteine		Total %	Total Iodine reduction as Glutathione, mg. %
		mg. %	% of Total iodine reduction	mg. %	% of Total iodine reduction	mg. %	% of Total iodine reduction		
				(*a*)[1]					
1	1	30.3	57.7	65.7	36.7	2.8	5.0	99.4	179
	12	23.2	56.6	54.9	39.2	3.5	8.1	103.9	140
2	9	18.5	58.3	26.3	24.2	7.9	23.5	106.0	109
3	11	28.0	58.6	26.3	15.5	11.2	21.3	95.4	169
Avg.		25.0	57.8	43.3	28.9	6.4	14.5	101.2	149
				(*b*)					
4	1	31.0	54.1	70.9	37.7	4.6[2]	6.2	98.0	188
5	6	27.1	52.0	58.7	34.3	5.9	8.7	95.0	171
	13	20.2	53.3	46.0	36.9	3.6	7.4	97.6	125
6	13	20.3	54.8	47.3	38.9	2.4	5.1	98.8	122
	20	7.8	45.3	31.3	55.6	0	0	101	56
Avg.		21.3	51.9	50.8	40.7	3.1	5.5	98.1	132

[1] Table (*a*) represents earlier and less perfect results than (*b*). For example, the glutathione method in (*a*) was an earlier variation of the present one. Table (*a*) is given here only because it was presented at the meeting of the Association for the Study of Internal Secretions at Atlantic City in June, 1937.
[2] Using Wratten filter No. 58.

the largest percentage (about 55 per cent) of iodine-reducing substances, with glutathione next (about 37 per cent), and cysteine the smallest (about 7 per cent). In such fresh extracts the concentrations of ascorbic acid, glutathione, and cysteine are about 30, 65, and 5 mg. per cent, respectively. The sum of the iodine reduction of the components is approximately equal (within the experimental error) to the total iodine reduction, indicating that no appreciable amount of other iodine-reducing material is present in the thymus extract. The fact that the total percentage is generally slightly below 100 per cent may possibly be explained by the total iodine reduction titration giving results slightly greater than strictly additive for the components.

Specificity of methods. It is obvious that the validity of the conclusion that the iodine-reducing material of the thymus extract is approximately accounted for by its content of ascorbic acid, glutathione and cysteine, rests upon the specificity of the methods used for their determination. In the first place, each method as used excludes the other two substances. Other naturally occurring material exists that would be measured by these methods. However, evidence will now be given that such material is not present in appreciable quantities in the thymus extract.

Evidence of non-ascorbic acid indophenol-reducing material in the liver has been reported by Roe (17) using his furfural colorimetric method for ascorbic acid. We have found that the Roe method agrees with the indophenol titration when applied to the thymus extract. The specificity of the indophenol method for the thymus gland has also been previously reported (5). Ascorbic acid has been found in the thymus extract by biological assay (18).

Cysteinyl glycine, a hydrolytic product of glutathione, is known (19) to react in the Sullivan cysteine method. However, evidence that most of the Sullivan reacting material of the thymus extract is cysteine rather than cysteinyl glycine is adduced by the fact that the color of the unknown matches closely with the cysteine color and not with the cysteinyl glycine color at about the same color intensity.

When a correction is made for the precipitation of cysteine, we assume that the glutathione method is specific as far as known tissue constituents are concerned. A preliminary attempt[2] was made to apply the Woodward glyoxalase method (20) for glutathione to the thymus extract, but because of its toxicity towards glyoxalase, possibly due to the chlorbutanol added, only positive qualitative results were obtained.

SUMMARY

In the thymus extract, active in accelerating growth and development in the offspring of rats, the iodine-reducing material has been analyzed. Ascorbic acid, glutathione, and cysteine, approximately account for all the iodine reduction; in fresh extracts their respective percentages of iodine reduction being about 55, 37, and 7, and their respective concentrations being about 30, 65, and 5 mg. per cent. Ergothioneine is absent.

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THE EFFECT OF AN ANTERIOR PITUITARY SEX FRACTION UPON THE DEVELOPMENT OF THE HUMAN UTERUS

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The importance of the interrelations between the pituitary and the gonads has become well established since Cushing's (1) demonstration that partial hypophysectomy in female dogs is followed by hypoplasia of the generative organs. Smith (2) and Zondek and Aschheim (3) by replacement studies showed that the anterior pituitary gland in rats secretes a substance essential to the functioning of these glands. In young birds, Mitchell (4) reported delay in sexual maturity following nearly complete ablation of the hypophysis and Domm (5) observed gonadal hypertrophy and marked development of other sexual characters after hypophyseal implants or injections of pituitary hebin in such birds. Marked hypertrophy of the vagina, uterus and ovaries of dogs injected with anterior lobe pituitary extract was found by Putnam (6).

That uterine growth and development to some extent depends upon ovarian function, and that uterine atrophy follows castration and menopause has long been known. Recently, Greene and Ivy (7) feminized male rats in utero producing growth and development of uteri, oviducts and upper vaginae by administration of estradiol dipropionate to the pregnant mothers. Also Hamilton (8) and others have produced development of the genitalia in retarded males by the injection of male hormone substance, and in a single case Janson (9) reported gradual enlargement of a small uterus in a 21-year-old woman on administration of 26,000,000 M.U. of progynon-B, pregnancy following shortly. However, although structural growth may be attained, there is some danger of depressing gonad function by the administration of sex hormone (10).

The reports of Engle (11), Evans (12) and others were followed by a deluge of clinical papers attesting the stimulating effect of APL on cryptorchism and male genital underdevelopment. Werner (13) and also Bigler (14) showed that gonadotropic extracts from the anterior pituitary gland had a similar beneficial effect in stimulating underdeveloped males. Although Hamblen (15) and others using APL and anterior pituitary extract in underdeveloped females with amenorrhea and oligomenorrhea noted symptomatic improvement in many cases, there is a dearth of clinical reports noting structural changes following such treatment.

This report comprises a group of 10 cases with small uteri (table 1) observed consecutively during a period of 12 months. All had some menstrual disturbance when first seen. Only cases with a uterine depth of less than 2

TABLE 1

Case	Age	Menarche	Type	Treatment				Menstruation		Uterus	
					Duration Weeks	Frequency	Total R.U.	Frequency	Char.	Depth in.	Endometrium phase
1 (F. N.)	23	13	Eunuchoid	thyroid	10	1–2 gr. daily		28–180	Scanty	1	Atrophic
				theelin		2000 I.U. 3× weekly		none		1	Atrophic
				thyroid	3	2 gr. daily					
				A.P.		25 R.U. 2× weekly	150	none		1	Atrophic
				thyroid	5	2 gr. daily					
				A.P.		50 R.U. 2× weekly	500	none		1	Atrophic
2 (D. H.)	19	12	Masculine	A.P.	20	25 R.U. 2× weekly	1000	28–180	Prolonged	1½	Atrophic
								31– 35	Average	1½	Arrested estrin
				A.P.	11	50 R.U. 2× weekly	1100	38– 45	Average	2½–3¼	Secretory
				none	26			27– 30	Average	3¼	Secretory
3 (E. M.)	21	14	Masculine	A.P.	13	25 R.U. 3× weekly	975	90–120	Scanty	1½	Atrophic
								38– 52	Scanty	1¾	Interval
4 (T. B.)	24	15	Feminine	thyroid	13	1–2 gr. daily		28– 56	Scanty	1½	Atrophic
				thyroid	9	2 gr. daily		30– 32	Average		Secretory
				A.P.		25 R.U. 2× weekly	450	30– 32	Average	1½	Secretory
5 (S. B.)	20	16	Eunuchoid	A.P.	14	25 R.U. 2× weekly	700	120–365	Average	1¾	Atrophic
								34– 61	Average	1¾	Interval
6 (A. R.)	26	16–17	Eunuchoid	A.P.	3	25 R.U. 3× weekly	225	28– 30	Scanty	1¾	Interval
										2	Pregnant
7 (F. C.)	23	14	Masculine	A.P.	13	25 R.U. 3× weekly	975	31– 32	Scanty	1¾	Atrophic
								23– 37	Average	2½	Early secretory
					13	25 R.U. 3× weekly	975	23– 37	Average	3	Secretory
8 (E. W.)	21	12	Neuro-pituitary	A.P.	9	25 R.U. 3× weekly	675	24– 28	Average	1½	Atrophic
								15– 31	Average		
				A.P.	8	25 R.U. 3× weekly	600	29– 31	Average	2¼	Secretory
				thyroid		½–1 gr. daily					
9 (R. K.)	32	17	Eunuchoid	A.P.	16	25 R.U. 3× weekly	1200	21– 42	Scanty	1½	Atrophic
								32– 39	Average	1½	Secretory
10 (M. M.)	29	12	Masculine	APL alternating every 2 wks.	12	1000 R.U. 3× weekly		6 yrs. amenorrhea		Small	
				Estrin		2000 I.U. 3× weekly		none		1	Atrophic
				A.P.	11	25 R.U. 4× weekly	1100	one period		1¼	Atrophic

inches were included in the series. The measurement 'uterine depth' is the greatest depth a uterine sound would pass in the uterine cavity measured from the cervical os. Although this measurement may not represent the true depth of the uterus, this seemed most convenient and reliable for a comparative study. Endometrial biopsies[1] were taken, usually the third to fourth week postmenstrually and, when possible, each week thereafter until menstruation occurred. Biopsy specimens taken 7 days or less before menstruation were designated 'pre-menstrual.'

[1] Microscopic slides were prepared by the Cottage Hospital (Santa Barbara) Laboratory through the courtesy of Dr. Franklin Nuzum.

A gonadotropic extract[2] derived from the anterior pituitary glands of either hogs or sheep was used in these experiments and for the purpose of abbreviation has been designated A.P. Each cubic centimeter contained 25 R.U. each unit representing that amount of hormone which will cause 100 per cent increase in ovarian weight when 26 to 28-day-old rats are injected twice daily for 3 days, the ovaries being examined and weighed 96 to 100 hours after having started injections. An exact comparison cannot be made between dosages of A.P. and pregnancy urine prolan (antuitrin-S) since the urine extract of pregnancy is assayed on the production of hemorrhagic follicles rather than whole ovary growth. For general guidance it has been suggested[2] that a ratio of 1 R.U. of the A.P. hormone is comparable to about 3 R.U. of the APL hormone.

CASE HISTORIES

Case 1. F.N. was a white divorcée, aged 23 years, para o-o-o. Her development was considered normal, with menarche occurring at 13 years and regular periods lasting 3 to 4 days at 30-day intervals up until an attack of mumps when 15 years of age. Six months' amenorrhea followed this illness, and menstrual periods have since been scanty, of 3 to 5 days duration at 1- to 7-months' intervals. Libido was absent; during 3 years of married life she had no orgasm.

Physical Examination. The patient had eunuchoid, boyish stature, her height was 64 inches, her weight 116 lb. Her optimal weight[3] was 118.5 lb., the hair fine and abundant, in female distribution except at the pubis where there was definite male escutcheon. Her breasts and external genitalia were well developed, the uterus small, the anterior depth being 1 inch, and the ovaries small. The general physical examination otherwise showed normal findings.

Laboratory. Her B.M.R. was —24 per cent and —20 per cent; the glucose tolerance: fasting 80; half-hour 121; 1 hour 109; 2 hours 91; 3 hours 62; 4 hours 95. A biopsy showed an atrophic endometrium.

Course and Treatment. The patient was given thyroid, 1 gr. daily for 2 weeks, then 2 gr. daily throughout the observation period. She received theelin, 2000 I.U. 3 times weekly for 10 weeks. No mense occurred. The uterus remained 1 inch in depth and the endometrium atrophic. She was then given injections of A.P., 1 cc. twice weekly for 3 weeks, followed by 2 cc. twice weekly for 5 weeks. No bleeding occurred. There was no change in the size of the uterus and no change in the endometrium.

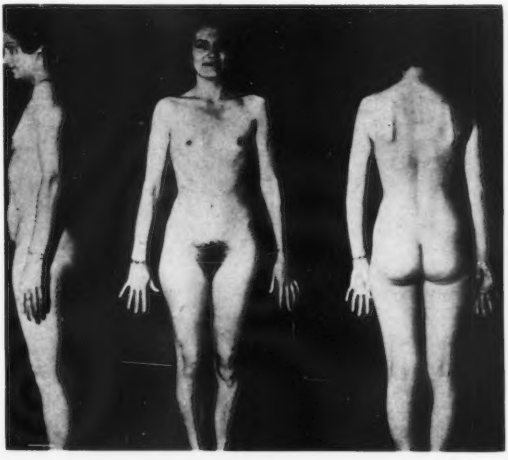
Case 2. D.H. was a white single female, aged 19 years, para o-o-o. Her development had been considered normal, with menarche occurring at 12 years, the menses occurring at 1 to 6 monthly intervals, usually at 28- to 35-day intervals, lasting 7 to 8 days with average flow.

Physical Examination. Her body contour suggested the masculine. The features were somewhat acromegalic, with protruding lower jaw and large hands and feet. Her height was 65.5 inches, her weight 121. Her optimal weight was 120.5 lb. There was a moderate hair growth over the legs and arms; otherwise the hair was of usual feminine distribution. The breasts were well developed and virginal, the external genitalia normal, the uterus small, with a cavity 1¼ inches in depth. The cervix and tubes were normal, the ovaries small. The general physical examination otherwise gave normal findings (see photograph).

[2] We are indebted to Dr. E. A. Sharp, Parke, Davis & Company for the generous supply of this material.
[3] 'Optimal weights' were calculated according to the method of Willoughby (16).

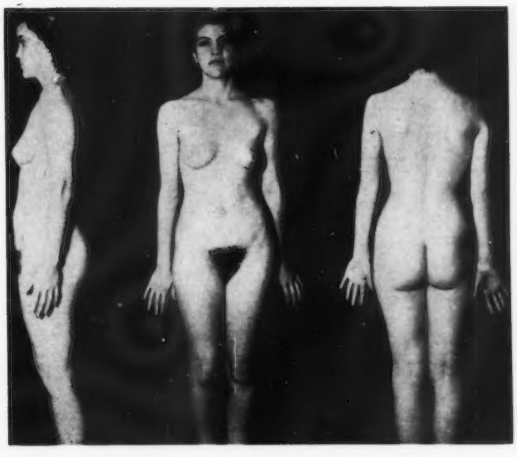
Laboratory. The B.M.R. was —15 per cent and —13 per cent, the glucose tolerance: fasting 89; half-hour 145; 1 hour 137; 2 hours 97; 3 hours 95; 4 hours 90. A premenstrual biopsy showed an atrophic endometrium.

Course and Treatment. The patient was given A.P., 1 cc. twice weekly for 20



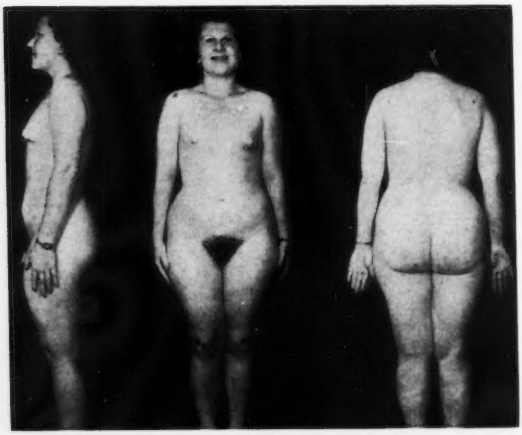
Case 2

This and the other cases pictured are those with infantile uteri which developed following treatment with A.P.

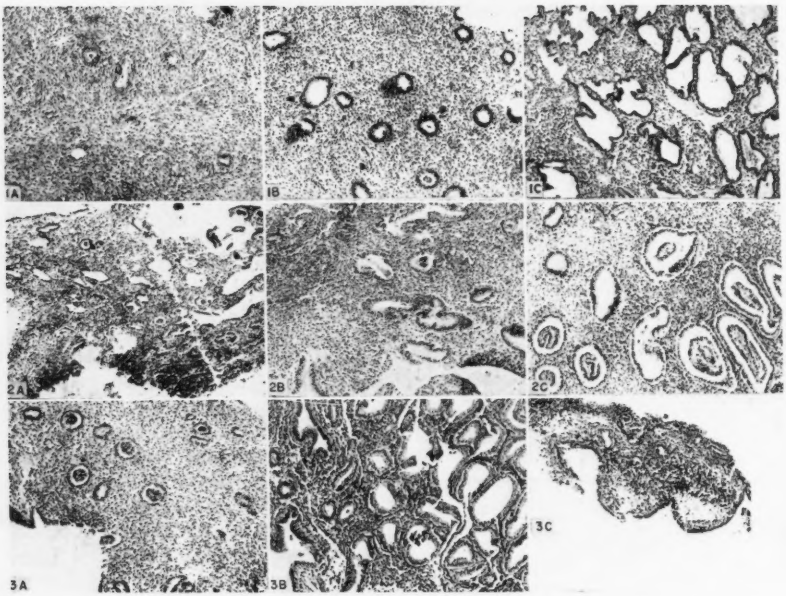


Case 7

weeks. Menstruation became more regular at 31- to 32-day intervals and of 5 days' duration. The uterus remained approximately the same size (depth 1½ in.) The premenstrual biopsy of the endometrium (fig. 1*A*) showed a thick edematous stroma, with only a very occasional tubular gland. Injections of A.P. were increased to 2 cc. twice weekly for 11 weeks. The subsequent menstrual period was delayed (45-day interval) and the flowing less profuse. The uterus increased in size (depth



Case 8



PHOTOMICROGRAPHS SHOWING CHANGE IN ENDOMETRIUM WITH TREATMENT

Fig. 1*A*. *Case 2*. After receiving A.P. twice weekly for 20 weeks. Premenstrual endometrium showing atrophic type.

Fig. 1*B*. *Case 2*. Endometrium 11 weeks later, A.P. having been increased to 2 cc. twice weekly. Twenty-seventh day endometrium showing an interval phase. A 'normal' menstrual period occurred 11 days later.

Fig. 1*C*. *Case 2*. Endometrium 6 months later, medication having been discontinued. Premenstrual endometrium showing normal secretory phase.

Fig. 2*A*. *Case 7*. Before treatment. Premenstrual endometrium (24-day) showing atrophic type.

Fig. 2*B*. *Case 7*. After receiving A.P. 1 cc. 3 times weekly for 13 weeks. Premenstrual endometrium (21-day) showing thin edematous stroma and early secretory phase.

Fig. 2*C*. *Case 7*. After receiving A.P. 1 cc. 3 times weekly for 26 weeks. Premenstrual endometrium showing thick edematous stroma and secretory phase.

Fig. 3*A*, *Case 8*. Before treatment. Premenstrual endometrial biopsy showing atrophic type.

Fig. 3*B*. *Case 8*. After receiving A.P. 1 cc. 3 times weekly for 17 weeks, thyroid also having been added during the latter 8 weeks. Premenstrual endometrial biopsy shows normal secretory phase.

Fig. 3*C*. *Case 10*. After A.P. 1 cc. on alternate days for 11 weeks. Endometrium atrophic.

2½ in.). The next period occurred after a 38-day interval and was of 4 days' duration and average flow. The uterus continued to enlarge (depth 3¼ in.). An endometrial biopsy (fig. 1*B*) taken 27 days following the previous period showed an interval type endometrium with thickened stroma and many small tubular glands. Medication was discontinued for 6 months. Menses continued at 27- to 30-day intervals lasting 5 to 6 days. The size of the uterus (depth 3¼ in.) remained unchanged. A premenstrual endometrial biopsy (fig. 1*C*) showed normal secretory type. There was no palpable change in the size of the ovaries.

Case 3. E.M., a white single female, age 21 years, para o-o-o, had always been thin and 'angular' since menarche at 14 years; her menses had been scanty, irregular and infrequent, usually occurring at 3- to 4-monthly intervals, lasting 7 to 8 days with severe dysmenorrhea the day before and first day of flowing.

Physical Examination. The patient was a small, thin female with masculine build; the shoulders broad, the thighs and hips narrow. The hair was of normal female distribution, the breasts fairly well developed. The extenal genitalia were well formed, the uterus small, with a depth of 1¾ in. The right ovary was cystic and twice the normal size; the left ovary and tubes were normal. The general physical examination otherwise gave normal findings.

Laboratory. Premenstrual biopsy showed an atrophic endometrium.

Course and Treatment. The patient received intramuscular injections of A.P., 1 cc. 3 times weekly for 13 weeks. Menses occurred at a 52- and a 38-day interval without change in character. The premenstrual endometrium showed an interval type; the uterus remained the same size (depth 1¾ in.).

Case 4. T.B. was a white single female, aged 24 years, para o-o-o. Menarche occurred at 15 years, with regular menses at 28- to 29-day intervals lasting 4 to 5 days until, while attending school at Berkeley 5 years ago, her periods became scanty, of 2 days' duration and occurring irregularly at 4- to 8-weekly intervals. At that time her B.M.R. was —13 per cent and she took thyroid (brand unknown), 1½ to 2 gr. for 4 or 5 months without noticeable effect.

Physical Examination. The patient was well developed, her height 63 inches, her weight 118 lb. Her optimal weight was 118.5 lb. The skin and scalp were dry, the hair luxuriant, in the usual female distribution except for marked hirsutism of arms and legs (shaved). The breasts were fairly well developed, the external genitalia normal, the uterus small, with a depth of 1½ inches. The tubes and ovaries were normal. The general physical examination otherwise showed normal findings.

Laboratory. Her B.M.R. was —27 per cent and —25 per cent. A premenstrual biopsy showed an atrophic endometrium.

Course and Treatment. The patient was given thyroid, 1 gr. daily for 2 weeks, then 2 gr. daily. Three normal menses followed at 30- to 32-day intervals. The premenstrual endometrial biopsy showed a normal secretory phase. A.P., 1 cc. twice weekly was added for 9 weeks without change in menstruation, premenstrual endometrium, or size of uterus (depth 1½ inches).

Case 5. S.B., a white single female, aged 20 years, para o-o-o, had always been tall and thin; had considerable orthodontial difficulty. Menarche began at 16 years, with menses occurring at 4- to 12-monthly intervals lasting 4 to 7 days. She used to have dysmenorrhea but during the past year this had been less. Both mother and maternal aunt had had late menarches.

Physical Examination. The patient was a tall, thin girl with eunuchoid stature, her height 69.8 inches, weight 110 lb. Her optimal weight was 139 lb. The hair was luxuriant, chiefly in feminine distribution, although there was a suggestion of

male escutcheon and moderate hirsuteness of the upper lip, arms and legs. There was slight diffuse enlargement of the thyroid. The breasts were fairly well developed, and so were the external genitalia; the vagina was long and deep, the uterus small, the uterine depth was 1¾ inches; the cervix was long; the tubes normal, and the ovaries small. The general physical examination otherwise gave normal findings.

Laboratory. The patient's B.M.R. was —12 per cent and —8 per cent, the glucose tolerance: fasting 90; half-hour 122; 1 hour 118; 2 hours 93; 3 hours 83; 4 hours 84. The sella turcica was normal. An endometrial biopsy showed atrophy.

Course and Treatment. The patient was given A.P., 1 cc. twice weekly for 14 weeks. The first mense occurred at 61 days, and a second 34 days later, both of 5 days' duration. The uterine depth was 1¾ inches. Biopsy of a premenstrual endometrium showed a thin dense stroma and several small tubular glands.

Case 6. A.R. was a white single female, aged 26 years, para o-o-o. Menses did not appear until the age of 16 or 17 years, and have since been fairly regular at 28- to 30-day intervals although very scanty, or 1 to 2 days' duration. For 7 to 8 years she has had a strong sapphistic urge, but there is no history of perversion. During the past year she has sought relief by indulging in heterosexual intercourse, but finds no enjoyment in this.

Physical Examination. The patient was a small, somewhat obese eunuchoid female, with many masculine characteristics. Her height was 59.1 inches, her weight 118 lb. The optimal weight was 98 lb. The hair was in usual female distribution. The breasts were pendulous and flabby, the clitoris large, the uterus small, with an anterior depth of 1¾ inches. The ovaries were normal. The general physical examination otherwise gave normal findings.

Laboratory. Her B.M.R. was —19 per cent and —11 per cent, the glucose tolerance: fasting 84; half-hour 111; 1 hour 106; 2 hours 90; 3 hours 84; 4 hours 87. The sella turcica was normal. A premenstrual endometrial biopsy showed a thin stroma and but a few tubular glands.

Course of Treatment. The patient was given intramuscular injections of A.P., 1 cc. 3 times weekly for 3 weeks. On routine examination the uterus seemed larger, with a depth of 2 inches. Treatment was discontinued. Three weeks later, pregnancy was confirmed and continued undisturbed in spite of the instrumentation.

Case 7. F.C., a white single female, aged 23 years, para o-o-o, had always been thin, in spite of efforts to gain weight. She was emotionally unstable, becoming excited on little provocation. Menarche occurred at 14 years, and was always fairly regular at 31- to 32-day intervals, of 1 to 2 days' duration and very scanty—"can hardly tell when period is present."

Physical Examination. The patient was a fairly well-developed girl, very anxious and querulous. Her shoulders were wide, the waist and hips relatively small. The extremities were long; the ligaments lax. Her height was 65.5 inches, weight 109.5 lb. The optimal weight was 116.4 lb. The breasts were undeveloped, the hair abundant, in the usual feminine distribution except for slight hirsutism over arms and legs. The skin was flushed and moist. The external genitalia were small. There was a cyst of the left Bartholin gland, 1 cm. in diameter. The uterus was small, 1⅜ inches in depth, the right ovary small, the left of normal size. The general physical examination otherwise showed normal findings (see photograph).

Laboratory. Her B.M.R. was —4 per cent and —3 per cent, the glucose tolerance: fasting 92; half-hour 126; 1 hour 108; 2 hours 96; 3 hours 82; 4 hours 80. A premenstrual endometrial biopsy (fig. 2*A*) showed a small amount of dense stroma and but a few tubular glands.

Course and Treatment. Patient received A.P., 1 cc. 3 times weekly for 26 weeks. Menstruation appeared at irregular intervals from 23 to 37 days, gradually increasing in amount and duration such that the third and subsequent periods were of 4 days' duration and saturating 3 to 4 napkins daily. After 13 weeks of treatment the uterus had enlarged (depth 2½ inches) and the premenstrual endometrium (fig. 2*B*) showed an edematous stroma, with few glands present, and some secretory change. She had no discomfort until the fifth and sixth periods which were accompanied by lower abdominal cramping, rather severe, and with nausea the first day of the fifth period, but less marked on the sixth period. At the sixth month the uterus was 3 inches in depth, the right ovary small and the left 1½ times normal size to bimanual palpation. Premenstrual endometrial biopsy (fig. 2*C*) showed a thick edematous stroma, the number of glands had increased and a good secretory effect was present. At the end of six months observation she weighed 118 lb. There had been no noticeable change in her excitability, and the breasts had not developed.

Case 8. E.W., a white single female, 21 years of age, para 0-0-0, had always been 'chubby.' The right breast failed to devolop. Manarche began at 12 years, menses appearing at 24- to 28-day intervals of 4 to 5 days' duration with average flowing. Prior to observation she had been taking 3 'thyro-ovarian' tablets daily for 4 months with no noticeable effect.

Physical Examination. The patient was short and moderately obese with generalized increase in fat padding, most marked over the hips and thighs, producing the 'high waist-line' effect. The hands were thick and short; the fingers blunt. Ligaments were relaxed, with a slight genu valgum. The skin was dry over the extremities, the hair fine and luxuriant, in the usual feminine distribution except for moderate hypertrichosis over the legs. Her height was 60.2 inches, weight 136 lb. Her optimal weight was 117 lb. The left breast was fairly well developed and virginal; the right undeveloped and flat. Blood pressure was 96/58. The external genitalia were normal, the uterus small, with a depth of 1½ inches. The cervix was tubular, the right ovary twice the normal size, prolapsed in cul-de-sac, and the left ovary and tubes were normal. The general physical examination otherwise gave normal findings (see photograph).

Laboratory. Her B.M.R. was —14 per cent and —5 per cent, the glucose tolerance: fasting 96; half-hour 117; 1 hour 100; 2 hours 94; 3 hours 78; 4 hours 84. A premenstrual biopsy of the endometrium (fig. 3*A*) showed a small amount of dense stroma and but few tubular glands.

Course and Treatment. The patient was given A.P., 1 cc. 3 times weekly for 17 weeks and, beginning the 9th week, also received thyroid, gr. ss. daily from the 9th to the 13th week, and 1 gr. daily the 13th through the 17th week. The first 2 menses were of the usual character but occurred at 15-day intervals. Menses have since occurred at 29- to 31-day intervals. Rather severe dysmenorrhea was present with the last period for the first time. The uterus increased in size (depth 2¼ inches) and the premenstrual endometrium (fig. 3*B*) showed many well-developed secretory glands. There was no noticeable change in the breasts. She lost 5.5 lb. during the 17 weeks' observation (weight 130.5 lb.).

Case 9. R.K., a white married female, aged 32 years, para 0-0-0, began menarche at 17 years. Menses always irregular at 3- to 6-weekly intervals, scanty and of 2 to 3 days' duration. She was married at 19 years of age, and intercourse had always been an 'ordeal.' She used no contraceptives during the past 8 years, as children were desired. She had been taking thyroid, 1 gr. daily for the past 11 months.

Physical Examination. The patient was a tall, thin eunuchoid female, with arms

and legs disproportionately long; hips narrow. Her height was 68.7 inches, weight 110 lb. Her optimal weight was 136 lb. The breasts were flat, the hair abundant in the usual feminine distribution. The skin was dry, the ligaments lax; with a moderate genu valgum. The external genitalia were small, as also the uterus (depth 1½ inches). The ovaries were small, the tubes not felt.

Laboratory. Her B.M.R. was —6 per cent and —12 per cent, the glucose tolerance: fasting 115; half-hour 156; 1 hour 115; 2 hours 111; 3 hours 97; 4 hours 82. The sella turcica was normal. A premenstrual endometrial biopsy showed an interval phase.

Course and Treatment. The patient was given A.P., 1 cc. 3 times weekly for 16 weeks. The first uterine bleeding occurred at a 36-day interval, and continued rather profusely for 9 days, with severe dysmenorrhea the first 2 days of flowing. Subsequent periods occurred at 39- and at 32-day intervals, lasting 4 days and with moderate flowing. There was no change in the size of the uterus (depth 1½ inches). A premenstrual endometrial biopsy showed abundant secretory glands.

Case 10. M.M., a white single female, aged 29 years, para o-o-o, whose early development was considered normal, began the menarche at 12 years. Her menses had always been infrequent, at 2- to 6-monthly intervals of 6 days' duration, and with moderate flow. She became very depressed following the death of her mother 6 years ago and complete amenorrhea followed. She had been given an unknown amount of thyroid for several months before observation, without subjective effect.

Physical Examination. The patient was masculine in build, although small-boned. Her height was 64.3 inches, weight 107 lb. The optimal weight was 110 lb. The hair was in the usual feminine distribution except for some hirsutism of upper lip, and rather marked hairiness over extremities (present since puberty). There was a slight diffuse enlargement of the thyroid. The breasts and external genitalia were fairly well developed, the uterus small (⅓ normal size), the cervix tubular, both ovaries pea-sized, and the tubes not felt. The general physical examination otherwise gave normal findings.

Laboratory. Her B.M.R. was —14 per cent and —12 per cent, the blood chemistry: phosphorous 2.5 mg./100 cc., serum calcium 9.8, serum cholesterol 115, glucose tolerance: fasting 100; half-hour 166; 1 hour 150; 2 hours 140; 3 hours 103; 4 hours 98. Creatin tolerance, first day: creatine 69; second day: creatine 918. Bioassays: 80 M.U. estrin, 5 C.U. androtin; 0 gonadotropin. x-rays showed a normal sella turcica.

Course and Treatment. For 12 weeks the patient received, 3 times weekly, alternate courses of P.U. prolan (follutein) and estrin, receiving 18,000 R.U. P.U. prolan and 36,000 I.U. estrogenic substance. No change was noted; the uterine depth was 1 inch, the endometrium atrophic. This treatment was discontinued and A.P. given, 1 cc. every other day. Thirteen days later she began having uterine bleeding, which continued for 8 days, requiring 2 to 3 napkins the first 3 to 4 days. There was rather marked lower abdominal cramping the entire 8 days. One month later she felt 'as if she were going to menstruate' but there was no bleeding. After 11 weeks of treatment with A.P. the uterine depth was 1¼ inches, but a biopsy specimen from the endometrium still showed atrophy (fig. 3*C*).

DISCUSSION

Underdevelopment of the internal female genitalia is not rare, and as more routine examinations are done more cases are discovered. As in the male, we have been accustomed to associate female genital underdevelopment with

eunuchodism, and lowered gonadal function manifest in the female by primary amenorrhea or delayed menarche and oligomenorrhea. However, only 3 cases of our series fall into this group, and small internal genitalia may be found not only in individuals with hypogonad habitus but also occur rather frequently in women with masculine build (4 cases) and in cases in which there are other gland disturbances. In many individuals, as in *case 6*, a small uterus may function quite adequately even to pregnancy, and many underdeveloped women may have no subjective signs which lead them to a physician.

Secondary menstrual disturbances occurred in 3 cases of our group: following an acute infection (*case 1*), a change in climate and environment (*case 4*) and after a severe nervous shock (*case 10*). During the postwar period in Germany, many young girls who had previously menstruated regularly became amenorrheic when removed from larger cities to rural districts. Wehefritz and Gierhake (17) found a striking prevalence of uterine hypoplasias in this group. Changes in gonadal function have been observed in the normal woman following such disturbances, and it may be that the inferiorly-developed women have a lowered threshold to such stress.

The endometrium obtained by biopsy at the beginning of treatment was atrophic in all patients. Since there was a response to treatment in all but 2 cases, it became apparent that we were dealing not only with a problem of uterine hypoplasia, but also with gonadal insufficiency. Since secondary sex characteristics were well developed in several cases, it seemed reasonable to assume that there had been some degree of gonadal activity at some time, and that the finding of atrophic endometrium in some cases was probably incidental.

Eight cases showed a response to treatment. In 7 cases there was improvement in menstruation and the endometrium became more normal following A.P. injections. All of these, except *case 8*, which was given some thyroid during the latter weeks, received A.P. alone. A.P. has an effect on the endometrium which may be interpreted as gonad-stimulating. Following injections of A.P. the endometrium changed to a secretory type in 4 cases, and one other became pregnant, suggesting that anterior lobe extracts may have an influence on ovulation. This is most impressive, since even with large injections of A.P.L. it has been impossible to produce ovulation (18) and only recently Davis and Koff (19) using intravenous injections of a gonad-stimulating substance, prepared from the blood of pregnant mares, for the first time have been able to produce ovulation in humans experimentally.

Case 4 responded well to thyroid alone, the menstrual periods and the endometrium approaching the normal. On addition of A.P., no further change was noted.

Uterine growth took place in 3 cases, the uterine depth increasing from 1¼ to 3¼ inches in *case 2;* 1⅜ to 3 inches in *case 7;* and from 1½ to 2¼ inches in *case 8*. Very little growth occurred in *case 2* until after 5 months of treatment, at which time the dosage was doubled. In all cases the dosage of A.P. used was small, and it is felt that if treatment had been more vigorous some of the cases reported as failures might have shown improvement.

Two cases (*1* and *10*) failed to respond to treatment. *Case 1* received 1 to 2 gr. thyroid daily, and also 60,000 I.U. of theelin during a period of 10 weeks. This was followed by thyroid, 2 gr. daily, and A.P. 750 R.U. during a period of 8 weeks. No uterine bleeding occurred, and there was no change in the uterine size or endometrium. During a 12-week period, *case 10* received 18,000 R.U. of APL and 36,000 I.U. of estrin. No change was noted; 1,100 R.U. of A.P. was given during the next 11 weeks. One period of uterine bleeding occurred 13 days after the first injection of A.P., but the endometrium remained atrophic and there was no change in the size of the uterus. Hormone assays in this case before treatment were within normal limits. Prolan, however, was absent and since this patient's body build was of the male type and the creatin excretion more like the male (20), the possibility that some androgenic substance was present and acting as an inhibiting factor was considered.

It is impossible to draw definite conclusions from such a small series of cases. However, it appears that the anterior pituitary elaborates a substance which is gonad-stimulating, including an influence on ovulation, and in some cases of uterine underdevelopment growth and development follows its administration.

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THE HYALINE CHANGE IN THE BASOPHIL CELLS OF THE PITUITARY BODY NOT ASSOCIATED WITH BASOPHILISM[1]

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In the 6 years since Harvey Cushing (1, 2) suggested an association between a striking clinical syndrome, which he designated pituitary basophilism, and a minute adenoma of the basophil cells in the anterior lobe of the pituitary body, there has been extraordinary interest in this subject. The clinical picture, when fully developed (3, 4, 5) consists of obesity of the face, neck and trunk, but sparing the extremities, rapid in onset and possibly accompanied by tenderness; hypertrichosis of the face and trunk among women and young males; amenorrhea among women; impotence among men; purplish striae from distention of the skin, especially in the lower part of the abdomen; a tendency to bruising; arterial hypertension with its various sequelae; polycythemia and dusky facies; diminished sugar tolerance, which frequently is associated with hyperglycemia and glycosuria; osteoporosis with resultant kyphosis and pain, and general bodily weakness.

This array of signs and symptoms has inspired clinical interest in this condition. In a recent review, Kessel (5) was able to collect 100 cases of basophilism. It is now clear that this syndrome may also be associated with hyperplasia or neoplasm of the suprarenal cortex, carcinoma of the thymus, arrhenoblastoma of the ovary, lesions in the region of the pineal body, and adenomas of the anterior lobe of the pituitary body that are composed of other types of cells than basophil. It also may occur without demonstrable adenoma or hyperplasia of any endocrine or other organ (5, 6, 7). However, endocrine changes are present clinically in only a small percentage of the cases of abnormal growths of any one of these glands. For example recent investigations (8, 9, 10, 11) have shown that adenomas of the basophil cells of the pituitary body occur not infrequently without previous clinical evidence of endocrine disease.

The tendency in the literature is to ascribe Cushing's syndrome to any large tumor of these endocrine organs which may be found and, especially in the absence of a gross lesion, to examine the pituitary body for minute lesions. Thus, of 35 fatal cases of this clinical state which he reviewed, Jonas (6) found the report of a basophil adenoma in 21, other types of adenoma in 5, a diffuse hyperplasia of the basophil elements in 4 and a normal hypophysis

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cerebri in 5 cases (in 3 of which the glands were studied in serial section).

In 1935, Crooke (12) suggested a common anatomic denominator for all cases of pituitary basophilism, namely, a specific hyaline change in the cytoplasm of the basophil cells of the anterior lobe of the pituitary body. This abnormality was first observed (13) in a case in which Cushing's syndrome was associated with a cortical carcinoma of the suprarenal gland; in this case there was no adenoma of the pituitary body. The large basophilic granules in the cytoplasm of the basophil cells seemed to have been largely displaced by a hyaline, agranular substance. In a later paper, Crooke (14) reported this change in 12 cases in which there was clinical evidence of pituitary basophilism, although careful postmortem examinations revealed a basophil adenoma in only half of the cases. He considered that, since this change was the only abnormality common to 12 cases, it was the abnormality of fundamental significance. In 1936, the number of cases of basophilism in which Crooke (14) was said to have found hyalinization of the basophils was increased to 29. This association has been confirmed by Rasmussen (15) in a case of pituitary adenoma and in one of a suprarenal carcinoma. The latter author has further supported the association of the hyaline change with the clinical syndrome of basophilism by finding the change in a case in which there was no adenoma or glandular hyperplasia (15) and also in one in which there was an arrhenoblastoma of the ovary (16).

As has been mentioned, in 6 of the first 12 cases of basophilism which Crooke reviewed, there was an associated basophil adenoma. He was unable to find the hyaline change in any of the cells constituting these adenomas, nor could he find it in 7 other basophil adenomas, 4 of which were observed in cases of high blood pressure. Indeed, in the literature there are only 2 instances which seem to represent hyalinization of the cells of a basophil adenoma itself. The first is the case reported by Graef, Bunim and Rottino (17), and even in this report the authors preferred to designate the type of the adenomas as 'indeterminate.' However, the description and drawing of the cells which they published seem to justify Crooke's considering these cells (which he had an opportunity to study microscopically) basophils in which the hyaline change had proceeded to an end stage. In any event, the authors described the basophil cells throughout pars distalis as characterized by enlarged, apparently lobulated nuclei and agranular, highly vacuolated cytoplasm; an occasional cell contained pyknotic nuclei, multiple nuclei, or evidence of amitotic division. They made the interesting suggestion that the cytoplasmic vacuoles seemed to have been extruded by the nucleus. Recently, Rasmussen (16) reported in a clinical case of basophilism, the finding of a basophil adenoma, an occasional cell of which presented some evidence of hyaline change, whereas the nonadenomatous basophil cells showed little evidence of such change.

Whether the change is physiologic or degenerative is still obscure, although the hyaline change has become generally accepted as a common anatomic denominator in all cases with basophilism (18). Likewise, there is the unsolved riddle as to whether it represents a primary dysfunction of the hypophysis cerebri or an effect secondary to disease of other organs.

Crooke (12) reported the study of the pituitary body in 350 cases in which basophilism was not present. In 9 of the cases he found a very slight hyaline change, which affected only a few of the basophil cells of the anterior lobe. He found, furthermore, that the change was absent in 7 cases in which a basophil adenoma of the pituitary body occurred without basophilism. Similarly, Rasmussen (15) examined sections of more than 200 normal pituitary bodies, and found only an occasional cell which was the site of a hyaline change.

The present study, which was made at the suggestion of Dr. J. W. Kernohan, represents another search for this hyaline change of Crooke in two series of pituitary bodies, all of which were obtained in cases in which there had not been any evidence of pituitary basophilism during the life of the patients. The first series was composed of pituitary bodies removed at routine necropsies; the other was a selected series of pituitary bodies which contained adenomas composed of basophil cells. This type of study is fundamental to the consideration of the relationship of Crooke's change to the clinical syndrome in question, to basophil adenomas of the pituitary body, and to the presence of tumors in other endocrine glands. However, it is beyond the scope of the present work to attempt such judgments.

MATERIALS AND METHODS

The material for this study consisted largely of that utilized by Costello (8) in a former investigation, that is, pituitary bodies obtained in the course of routine necropsies in which permission to examine the brain had been granted. Each pituitary body had been removed by breaking off the posterior clinoid processes with bone forceps and removing the body surrounded by its fibrous coat. It was then placed in a 10 per cent solution of formalin and stored.

In preparing the sections, the bodies were removed from the formalin and cut into sections paralleling the longest axis of the gland. Some of these blocks were then placed in Zenker's fluid for 24 hours whereas others were placed in Weigert's first and second mordants (19, 20, 21). After dehydration, they were embedded in paraffin in the usual manner. Sections, either 5 or 8 μ thick, were cut from these blocks and were stained by the modification of Mallory's acid fuchsin aniline-blue method which was recommended by Crooke and Russell (22) and which Crooke (12) used in his study. With this stain the cytoplasm of the chromophobe cells either does not stain at all or is pale blue; the eosinophil granules stain bright red and the basophil granules stain dark blue, whereas the hyaline substance uniformly stains pale blue. The preliminary staining with hemotoxylin, which Rasmussen recommended for distinguishing the nuclei, tends to cause the basophil granules to dissolve a little and to be smudged. Accordingly, this step was omitted in the study of the routine material. Even so, in those tissues which were fixed in Zenker's fluid the nuclei were clearly defined.

In pituitary tissues which have been in formalin for many years before being cut and embedded, the granules of the basophilic cells do not stain clearly. Accordingly, there were excluded from this study all pituitary bodies

which had remained in formalin too long. Therefore, a total of 721 supposedly normal tissues which were adequately prepared were available for the first part of this study. The 72 basophil adenomas which Costello (8) had previously reported were utilized for the second part of the study. For the latter, both Heidenhain's (19) and Crooke's (22) modifications of Mallory's stain were used. In reviewing the material, it was found that 54 of the first group of pituitary bodies, that is, those removed routinely at necropsy, happened to contain basophil adenomas; these were studied again and included with the second group of pituitary bodies selected because of the presence of basophil adenomas.

Each slide was examined with the low power (16 mm.) objective of the microscope and then several areas containing basophil cells were reëxamined with the oil-immersion objective and a very bright source of illumination, for the presence of the hyaline change. In the cases of basophil adenoma, several groups of cells both within and outside of the adenomas were studied in each slide. Of the sources of error in judging this change, one, namely, excessive fixation, has already been mentioned. Others result from improper staining. Overstaining with Mallory's aniline-blue mixture causes the cytoplasm of the chromophobe cells to appear more blue than usual. Conversely, understaining makes it more difficult than usual to study the basophil granules.

Masses of 'colloid' may resemble the completely hyalinized cytoplasm of a basophil cell but are distinguishable by the absence of nuclei. Furthermore, the thin rim of ectoplasm in a basophil cell normally contains no granules and must not be confused with an early hyaline change. Likewise, part of the cytoplasm of a chromophobe cell, the nucleus of which is in a different plane of the section, may give the erroneous impression of being an agranular area in the otherwise granular basophil cell.

As controls, there were available sections of pituitary bodies which were studied by Crooke (22) in cases previously reported by Kepler (23, 24) and by Walters, Wilder and Kepler (25) including one case of basophil adenoma of the pituitary body [case *5*, (24); case *2*, (23); case *9*, (25)] one case of neoplasm of the thymus and hyperplasia of the suprarenal glands [case *9*, (12); case *4*, (23); case *6*, (25)] and one case of hyperplasia in which the suprarenal cortices were associated with two minute (3 mm. and 4 mm. diameter) cortical adenomas in the left suprarenal gland [case *10*, (12); case *1*, (24); case 7, (25)]. As Crooke (12) has reported, in each of these cases of basophilism, the cytoplasm, which normally is filled with basophil granules (fig. 1*A*), had been replaced by a dense homogeneous hyaline cytoplasm in most of the basophil cells of the anterior lobe (fig. 1*B*). However, as Rasmussen (15) has pointed out, there also was more vacuolization of the cytoplasm than usual. The nuclei of these cells were enlarged and lobulated, as has been emphasized by Severinghaus (26). The vacuoles frequently were situated near the lobulated nucleus and give the impression, as in the case mentioned previously (17), of having been extruded from the nucleus. Furthermore, an occasional cell had 2 or 3 nuclei, and there was a definite tendency for the basophil cells to be larger than normal in all 3 cases. Even though Crooke

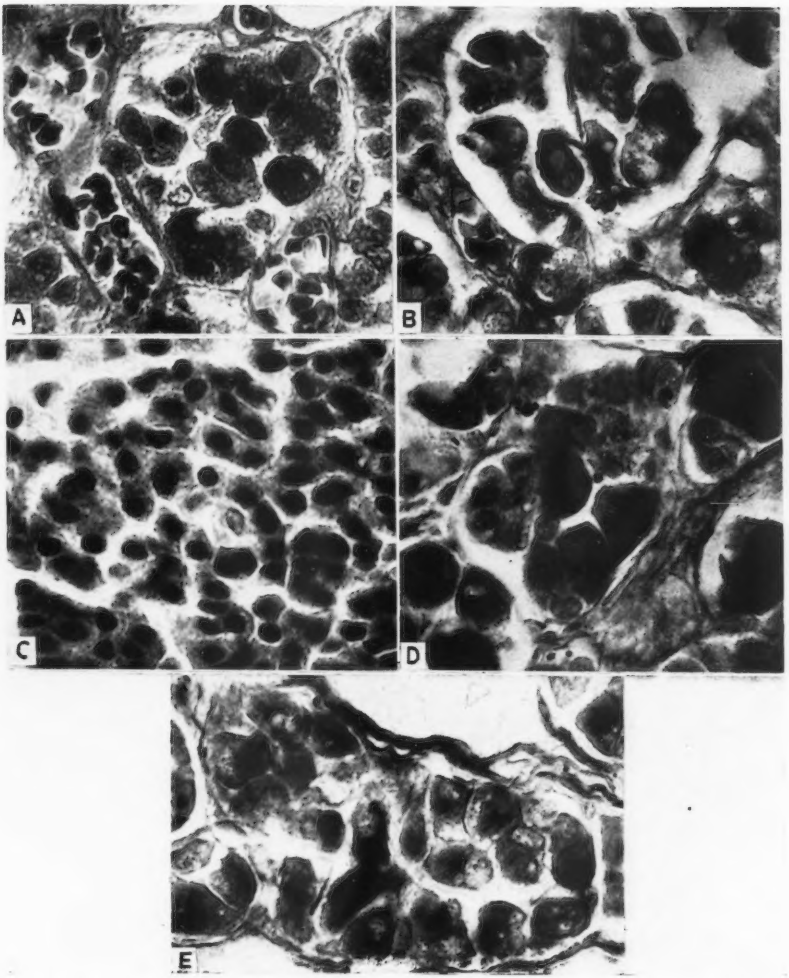

Fig. 1. *A*, Normal anterior lobe of pituitary body; acinus composed largely of basophil cells; the large, deeply staining granules partially obscure the nuclei; specimen stained with Crooke's stain (x605; yellow green filter). *B*, Pituitary basophilism; hyalinized vacuolated cytoplasm and with ballooned nuclei, specimen stained with Crooke's stain (x605; yellow green filter). *C*, Basophil adenoma in a recognized case of basophilism; the small size of the cells and the agranular blue cytoplasm with resultant clarity of the nuclei are noteworthy; specimen stained with Crooke's stain in Crooke's laboratory (x605; yellow green filter). *D*, Hyaline change without basophilism; the nuclei are normal and the basophil granules absent; a fine stippling in the cytoplasm is brought out in the photograph (as in the control, fig. *B*) but this is not seen under the microscope; specimen stained with Crooke's stain (x605; yellow green fiber). *E*, Hyaline change in the cells of a basophil adenoma not associated with basophilism; the large, deeply staining basophil granules are absent, although there is the fine cytoplasmic stippling seen in figures *B* and *D*; the nuclei here are larger than normal and have a minimal tendency to lobulation; specimen stained with the Mallory-Heidenhain stain (x605; yellow green filter).

denied that there was hyalinization in the cells of the basophil adenoma itself, some of the cells clearly do not have a normal structure (fig. 1*C*); the normal large granules which stain deeply are absent, the nuclei are clearly visible, and the cells are much smaller than normal.

RESULTS

A). In 8 of the 721 pituitary bodies studied, according to the method previously outlined, a marked degree of hyaline change was found (fig. 1*D*). The exact percentage of the cells thus affected was not determined. However, the requirements for tissue to be placed in this group were partial hyalinization of most of the basophil cells studied and extreme replacement of the granules in several cells. In none of these 8 cases was there ballooning of the nuclei, although they seemed slightly larger than normal in 3 cases. In tissue obtained from 3 cases (the patients were middle-aged persons) there was a little more cytoplasmic vacuolization than normal. This finding is in agreement with the observations of others (16, 27) that more vacuolization occurs in older individuals. In no instance was there the extreme degree of vacuolization which characterized the basophil cells of the control series. There was practically no tendency for the basophil cells to have more than one nucleus apiece and in no case were the cells markedly enlarged. However, in 3 cases the nucleoli did appear somewhat larger than normal. Rasmussen assisted with the microscopic studies.

The clinical histories and post-mortem protocols in these 8 cases were reviewed. Seven of the patients were males and one was a female; the ages of the patients varied from 13 to 55 years. The blood pressure in one case was 154 mm. of mercury systolic and 70 mm. diastolic; in all the other cases it was well within normal limits. In 3 cases the cause of death was meningitis and, in the others, ruptured aneurysm of the middle cerebral artery, gunshot wound of the spinal column, thrombosis of the lateral sinus, adamantinoma cerebri, and heat exhaustion. The only endocrine manifestation present in any of the cases was Fröhlich's syndrome, which occurred in the case of adamantinoma; this was the only case in which obesity was present and the only case in which the necropsy was limited to the head. The suprarenal glands were not enlarged in any of the 7 cases wherein a complete necropsy was performed, although minute cortical adenomas were present in 2. There were 2 cases in which the weight of the heart exceeded 355 gm. The thyroid gland was not abnormal in any of the cases studied. There was no mention, either in the clinical records or in the protocols, of striae distensae or of hirsuties; however, there was diminished hair in the case of dystrophia adiposogenitalis. Likewise, there was no record of impotence, amenorrhea, or osteoporosis. The erythrocyte count was recorded in 7 cases; it did not exceed 4,800,000 per c.mm. of blood in any of these cases. In 1 case of streptococcic meningitis in which the erythrocyte count was 4,400,000 per c.mm. of blood, the patient, who was a man aged 35 years, did have a dusky, cyanotic face. Glycosuria was not noted in the 4 cases in which reports of urinalysis were available. Likewise, there was no record that any of the patients had suffered unduly from bruising. In brief,

the necropsy and clinical records did not reveal any evidence of the clinical syndrome of basophilism.

It is true that in 1 of these cases the anterior lobe of the pituitary body also contained two small adenomas. One of these consisted of a mixture of chromophobe and basophil cells, and the other was composed solely of basophil cells. However, there was no hyalinization of the cytoplasm nor other abnormality of any of the basophil cells of these adenomas. How perfectly a diagnosis of pituitary basophilism would have been justified in this case, had there been a clinical suggestion of such a state!

B). Although there are as yet only 2 published reports (16, 17) of the apparent finding of the hyaline change of Crooke in the cells of a basophil adenoma of the pituitary body, it was thought advisable to make a special study of the 72 basophil adenomas which were found by Costello. Both the cells of the adenomas as well as the other basophil cells in the anterior lobes were studied by the method that has been outlined.

There were 4 basophil adenomas (5.5 per cent) in which both the Mallory-Heidenhain stain and Crooke's stain revealed hyalinization of the cytoplasm to an advanced degree (fig. 1*E*). The loss of granules was neither so complete nor so widespread as it was in the cells referred to in *A*. However, the cells of the 4 adenomas had enlarged nuclei, but there was only a slight tendency to lobulation and enlargement of the nucleoli. There was a minimal tendency to multinucleation in the cells of 2 of the adenomas, but neither excessive vacuolization nor enlargement of the cells as a whole was noted. In the non-adenomatous basophils in these 4 cases there was no evidence of hyaline change of the cytoplasm.

The clinical histories and post-mortem protocols in the 4 cases were reviewed as they were in the previous group. Two patients were males and 2 were females; the ages of the patients ranged from 23 to 57 years. The causes of death were traumatic shock with laceration of the heart, encephalitis with localized encephalomalacia, acoustic neurofibroma, and exhaustion from pernicious vomiting of pregnancy. There was only one case of obesity; in this case the patient was a woman who had undergone one complete and one partial oophorectomy and had suddenly gained 60 lb. (27.2 kg.) following the second operation, even though her menstrual periods had continued. Except for the facts that she was obese (she weighed 214 lb. or 97.1 kg., and her height was 161 cm.) and that her blood pressure at one reading was 140 mm. of mercury systolic and 95 mm. of mercury diastolic (the weight of her heart was 372 gm.) she had no evidence of basophilism. One of the other 3 patients had a blood pressure reading that was exactly the same, but the other 2 had a normal blood pressure. The skin of all 4 patients was normal except that the patient with the neurofibroma had several pigmented moles; none of the cutaneous manifestations of basophilism was present. Except for the presence of minute cortical adenomas in 1 case, which is a common finding at necropsy, the adrenal glands were normal, in both gross and microscopic appearance. The erythrocyte counts and the results of roentgenologic examinations of the skull were normal in the 3 cases in which the reports of these tests were available. Glycosuria

was present in only 1 case, that is, in the case of the pernicious vomiting, but the value for the blood sugar was normal (0.80 mg. per cent). In brief, the clinical histories and postmortem protocols revealed no evidence of basophilism in any of the 4 cases.

SUMMARY

The basophil cells in the anterior lobes of a series of 721 pituitary bodies which were removed routinely at necropsy were examined carefully for the hyaline change which Crooke (12) has described, and has considered the sole common anatomic denominator in cases in which the clinical syndrome, pituitary basophilism, is present. Fifty-four of the pituitary bodies contained basophil adenomas. The hyaline change was found to be present to a marked extent in eight of the pituitary bodies, an incidence of 1.1 per cent. However, the clinical and post-mortem records revealed no evidence of the syndrome in question, even though in 1 case a basophil adenoma (the cells of which, however, presented no evidence of hyalinization) also was present. The cells of the 54 basophil adenomas in this series, as well as of 18 others, representing a total of 72 adenomas previously found by Costello, were similarly examined. The hyaline change was present to a considerable degree in 4 cases (5.5 per cent). However, in these cases, there was neither hyalinization of the basophil cells outside of the adenomas nor any evidence of Cushing's syndrome in the clinical and post-mortem records.

Finally, in none of the instances of hyalinization of the cytoplasm in the basophil cells in the 12 cases referred to were there such additional features as ballooning of the nucleus (26), excessive vacuolization (15), a tendency to multinucleation, and general enlargement of the cells, which characterized the basophil cells of the pituitary body in 3 well-authenticated cases (25) of pituitary basophilism which were used as controls. It appears, therefore, unwarranted to consider hyalinization or degranulation of the basophil cells in the anterior lobe of the pituitary body of the human as invariably associated with the state designated as pituitary basophilism. Whether the hyaline change, when found together with the other cytologic features mentioned, will prove to be the "alteration of fundamental significance in the causation of this syndrome" (12) is an open question.

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COMPARATIVE EFFECTS OF LIGHT STIMULATION AND ADMINISTRATION OF GONADOTROPIC HORMONES ON FEMALE SPARROWS[1]

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Poultrymen have known for a long time that artificial lengthening of the day during the winter stimulates ovarial development and egg laying. Experimental evidence of induced egg production has been published for the duck by Benoit (1); for the dove by Cole (2) and for pheasants and quail by Bissonnette and Csech (3). There have been no precise data published concerning the response of female passerine birds to light treatment. Rowan (4) finds some follicular development in the ovaries of juncos. Bissonnette (5) remarks that female starlings respond positively. As yet, however, he has not described clearly the extent of the reaction. Ringoen and Kirschbaum (6), in an abstract, mention that in the sparrow a "significant enlargement of ovary and oviduct" is obtained only after 70 or more days of stimulation (when the experiment is begun in November). Light-induced egg laying out of season has never been reported for any wild passerine bird.

MATERIAL AND METHODS

In our light experiments 6 groups of female sparrows (*Passer domesticus* Linnaeus) were subjected to increased daily light periods, each group being started at a time progressively closer to the normal breeding season. Beginning with sunset on the first day of each experiment, a 100-Watt incandescent bulb was turned on for an additional half-hour each day, this added light being extended until the hour of 1:00 A.M. was reached. From this time until the termination of the experiment, the daily light periods remained uniform. Exploratory laparotomies were performed preceding each experiment to determine the condition of the ovaries. The gonadotropic preparations used were beef pituitary extracts, horse pituitary extract, turkey pituitary powder and pregnant mare serum.[2]

The condition of ovarial and oviduct development attained by light-treated and hormone-stimulated birds is indicated by reference to the 4 stages illustrated in figure 1.

Exposure to Additional Daily Light Periods

The five birds in the first group (table 1) were exposed to lengthened daily light periods for 61 days (starting October 8). Three birds gave some response, small follicles appearing on the surface of the ovary with convolution of the

[1] Aided by grants from the National Research Council, Committee for Research in Problems of Sex.
[2] We are indebted to Dr. Kamm of Parke, Davis & Company, and Dr. Hart of the University of California at Davis, for the supply of these sera.

posterior part of the oviduct. The ovaries of the other 2 birds remained unchanged. The 2 birds started in the last week of November both gave definite positive responses after only 24 days' exposure. The majority of the females of the 3rd, 4th and 5th groups gave minimal responses, although in several instances there was no observable effect. The 4th group is of special interest. Five females were subjected to the light treatment for a total of 75 days. In spite of this long period of exposure the ovaries and oviducts had not advanced beyond Stage I. That the gonads of these birds were in a responsive condition was subsequently shown by the injection of 3 of them with pregnant mare serum (table 4, *group 8*). In the 5th group an activated (light-stimulated) male was

TABLE 1. EFFECT OF ADDITIONAL DAILY LIGHT PERIODS

No. of Females	Time of Treatment		Reaction (No. of cases)	
	Date	No. of Days	Ovary[1]	Oviduct
5 exposed	Oct. 8–Dec. 7	61	0(2), I(3)	0(2), I(3)
2 controls	Oct. 8–Dec. 7	—	0(2)	0(2)
2 exposed	Nov. 27–Dec. 19	24	I(2)	I(2)
18 controls	Nov. 27–Dec. 19	—	0(18)	0(18)
7 exposed	Jan. 5–Feb. 1	28	0(3), I(4)	0(3), I(4)
2 controls	Jan. 5–Feb. 1	—	0(2)	0(2)
5 exposed	Jan. 8–Mar. 24	75	I(5)	I(5)
3 controls	Jan. 8–Mar. 24	—	0(1), I(2)	0(1), I(2)
3 exposed	Feb. 8–Mar. 1	21	0(1), I(2)	0(1), I(2)
4 +male	Feb. 8–Mar. 1	21	I(4)	I(4)
3 +disturbance	Feb. 8–Mar. 1	21	I(3)	I(3)
2 controls	Feb. 8–Mar. 1	21	0(2)	0(2)
7 exposed	Mar. 20–May 15	57	I(7)	I(7)
3 controls	Mar. 20–May 15	—	II(2), I(1)	II(2), I(1)
10 newly caught	Killed Apr. 19	—	III(7), II(3)	III(7), II(3)

[1] O, I, II and III refer to the stages of ovarian and oviduct development illustrated in figure 1. The small figures in parentheses indicate the number of cases.

placed in the cage with 4 females to test the possibility of the presence of an essential psychic factor. Three additional birds were placed in a cage automatically shaken during the light treatment to insure a permanent state of awakeness.

With the approach of the normal breeding season a more pronounced effect was expected. However, the ovaries and oviducts of the 7 females subjected to lengthened daily periods from March 20th to May 15th (total of 57 days) were no further advanced than in control birds, in fact, the ovarial development of two of the controls exceeded that of the most advanced experimental bird. This lack of ovarial development in birds exposed to additional illumination at this time of the year markedly contrasts with the situation in nature, where most females are found in full breeding condition by the middle of April (fig. 2).

Injections of Gonadotropic Substances

Since longer days were inadequate to bring about the breeding condition, an effort was next made to determine whether the non-effect was due to non-

responsiveness of the anterior lobe to the external stimulus or to a refractoriness of the ovary. Our first experiments seemed to indicate that the ovaries, like testes, responded at all times to injections of moderate amounts of hypophyseal preparations and pregnant mare serum (7, 8). In later experiments, it was found exceedingly difficult to stimulate ovaries in September and October, even when relatively large amounts of gonadotropic substances were injected.

TABLE 2. EFFECTS PRODUCED BY BEEF AND HORSE PITUITARY EXTRACTS

No. of Females	Approx. Am't. Inj.		Time of Injection		Reaction	
	D.R.U.	Total R.U.	Date	No. of Days	Ovary	Oviduct
1	2–3	48–72	Oct.–Nov.	24	III	III
3	1	24	Oct.–Nov.	24	0(2), I(1)	0(2), I(1)
1[1]	2	32	Dec.	16	III	III
1[1]	2	46	Dec.	23	III	III
1	3F	39	Apr.	13	III	III
1	3F+3L	39F+39L	Apr.	13	III	III
1	3L	33L	Apr.	11	II	II

[1] Received horse pituitary extract.

Injection of beef and horse pituitary extracts. Maximal responses of the ovaries to injections of beef and horse anterior lobe extracts were obtained from October to April (table 2). Only a few birds were used at various times of the year, and consequently the chance for individual variations to appear was small. That differences in degree of responsiveness may exist is suggested in the group of 3 birds receiving a total dosage of 24 R.U. Two of the 3 birds gave

TABLE 3. EFFECTS PRODUCED BY INJECTIONS OF TURKEY PITUITARY

No. of Females	Approx. Am't. Inj.		Time of Injection		Reaction (No. of cases)	
	D.R.U.	Total R.U.	Month	No. of Days	Ovary	Oviduct
2 a.[1]	0.5	4	Sept.	9	0(2)	0(2)
4 j.[1]	0.5	8	Oct.	16	0(2), I(1) II(1)	0(2), I(1) II(1)
7 (+light)	0.5	6	Dec.	12	0(2), I(2) II(2), III(1)	0(2), I(2) II(2), III(1)
7	0.5	6	Dec.	12	I(1), II(4) III(2)	I(1), II(4) III(2)
2 (+light)	0.5	3	Feb.	6	II(2)	II(2)
1	0.5	3	Feb.	6	II(1)	II(1)
2	0.5	6	Feb.	12	III(2)	III(2)
2	0.25	4	Feb.	15	II(1), III(1)	II(1), III(1)
1	0.5	5	Feb.	11	III(1)	III(1)

[1] a. = adult females; j. = juvenile females.

negative responses. That the one bird receiving the luteinizing fraction of an extract of beef anterior lobe responded at all is due, in all probability, to contamination of the extract with the follicle-stimulating principle.

Injection of turkey pituitary powder. In February, 1937, 3 females (last two groups in table 3) were injected with turkey pituitary powder which, in assays on immature rats, was found to produce follicular growth and luteinization (9). These 3 birds gave maximal or near maximal responses. In the fall of the

same year (*groups 1* and *2*, table 3), of 6 birds receiving from 4 to 8 total R.U., 4 remained at Stage 0 and the other two also failed to reach the breeding condition. It is of interest to note that both adult and juvenile females respond poorly, or not at all, at this time of the year.

In the third group, 7 females were subjected to lengthened light periods 10 days before and during the course of the injections. Differences in individual reactions ranged from Stage 0 to the full breeding condition. The 7 females receiving only the injections showed considerable variation in response but, in general, development was better.

Of the first 5 females (two receiving light as above) injected in February, 3 died after 6 daily injections. However, all ovaries had developing follicles

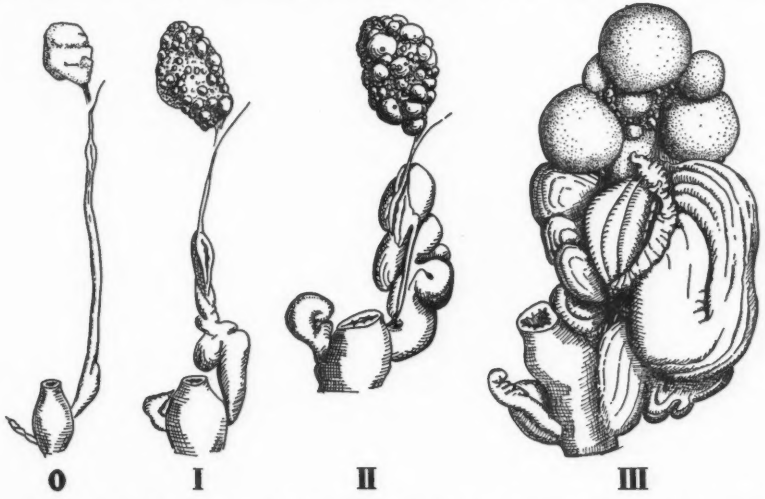

Fig. 1. Four developmental stages of ovaries and oviducts in the domestic sparrow. Stage 0 illustrates the quiescent condition in a control female; weight of ovary ca. 9 mg. Stage I, condition in a female which received a total of 15 R.U. of pregnant mare serum (in 15 days); weight of ovary 27.0 mg.; enlarged 3 times. Stage II, condition in a female which received a total of 68 R.U. of pregnant mare serum (in 17 days); weight of ovary 39.2 mg.; enlarged 4 times. Stage III, condition in a female which received a total of 340 R.U. of pregnant mare serum (in 17 days); weight of ovary 288.8 mg.; enlarged 30 times. x2.5.

and the oviducts were considerably enlarged. The remaining 2, injected for 12 days, were stimulated to full breeding condition.

Injection of pregnant mare serum. On the basis of total rat units injected, it will be observed (table 4) that much higher dosages of pregnant mare serum are necessary to elicit the maximal response in female sparrows than hypophyseal extracts or powders. When moderate dosages are given, wide differences in individual responsiveness to pregnant mare serum persist even as late as February. At this late point in the inactive phase of the cycle, full breeding condition is attained with high dosages.

Supplementing injections with light treatment during November, December and February does not reduce the variability, nor does it perceptably augment responsiveness. The marked individual variability in response which may exist is strikingly illustrated in the fourth group of females in table 4. Two birds treated with light and receiving a high dosage (240 total R.U.) of

pregnant mare serum gave no significant response. On the other hand, one of the 3 birds not treated with light and receiving only 24 total R.U. developed to the breeding condition.

DISCUSSION

That lengthened daily light periods during the winter can activate the female pituitary, seems definitely established. However, in contrast to the extensive development resulting in egg production in such forms as the domestic duck, game birds and the pigeon (all of which normally breed in captivity), the reaction in wild passerine birds is limited. From descriptions in the literature it is impossible to say whether the females used in the present investigation were

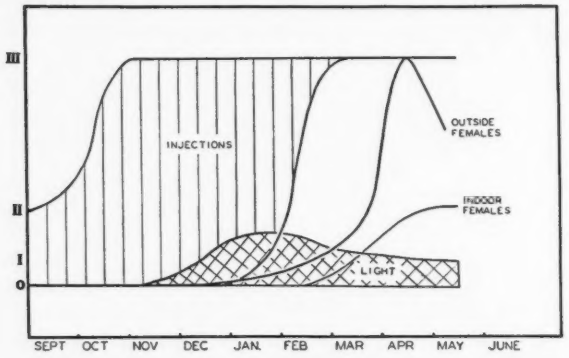

Fig. 2. Graphic illustration of gonad development in the female sparrow under normal and experimental conditions. The 4 stages on the ordinate correspond to those represented in figure 1. The curve for 'outside' females is based on the average stage of development observed in newly-caught females at various times of the year. The curve for 'indoor' females is based on the stage attained by untreated females in the laboratory. The cross-hatched area represents the extent and variability of response to lengthened daily light periods; the lined area, the period during which variability in response to gonadotropic substances occurs.

less responsive than the passerine females used by other workers. It does not seem improbable, however, that birds of other species, or birds of the same species in different geographical locations, may show differences in reactivity to light treatment.

That a dietary deficiency does not account for the low-grade reaction in females is indicated by the good responses of females injected with various hormones and the marked gonad stimulation in male sparrows kept on the same diet (cracked corn). After 3 weeks of light treatment, testes are enlarged and often mature spermatozoa are found in the seminiferous tubules (10, 11). In nature, the males are in the breeding condition by the middle of March, whereas the females do not reach this stage until approximately a month later activity of the anterior lobe of the bird pituitary is behavioristically conditioned (fig. 2). It would seem reasonable to expect, therefore, that a longer period of exposure to artificially lengthened days would be necessary for stimulation of the female; yet periods of 57, 61 and 75 days were no more effective than shorter periods. That some factor in addition to lengthened light periods is necessary is further indicated by the reaction of untreated females kept in the laboratory. These birds have never been observed to advance beyond Stage II,

TABLE 4. EFFECTS PRODUCED BY PREGNANT MARE SERUM

No. of Females	Approx. Am't. Inj.		Time of Injection		Reaction (No. of cases)	
	D.R.U.	Total R.U.	Month	No. of Days	Ovary	Oviduct
1	4	68	Oct.	17	0	0
1	8	136	Oct.	17	I	I
6	4/5	14	Nov.	17	0(4), I(2)	0(4), I(2)
5 (+light)[2]	4/5	14	Nov.	17	0(5)	0(5)
2	2	20	Dec.	10	0(2)	0(2)
1	20	200	Dec.	10	0	0
2 (+light)[3]	2	20	Dec.	10	0(1), I(1)	0(1), I(1)
1 (+light)[3]	20	200	Dec.	10	III	III
3	2	24	Dec.	12	0(2), III(1)	0(2), III(1)
3	20	240	Dec.	12	0(1), II(1), III(1)	0(1), II(1), III(1)
3 (+light)[2]	2	24	Dec.	12	0(1), II(1), III(1)	0(1), II(1), III(1)
2 (+light)[2]	20	240	Dec.	12	0(2)	0(2)
1 (+light)[2]	20	140	Dec.	7	I	I
3 (+light)[2]	2	24	Feb.	12	0(1), I(1), II(1)	0(1), I(1), II(1)
3	2	24	Feb.	12	I(1), II(1), III(1)	I(1), II(1), III(1)
3	20	340	Feb.	17	III(3)	III(3)
1	40	520	Feb.	13	III	III
2	1	17	Mar.	17	0(1), I(1)	0(1), I(1)
3	4	68	Mar.	17	I(3)	I(3)
3 (+light)[1]	20	240	Mar.–Apr.	12	III(3)	III(3)
3	20	500	Apr.	25	III(3)	III(3)

[1] Subjected to light treatment for 75 days preceding injections.
[2] Light treatment for 10 days preceding and during course of injections.
[3] Light treatment during period of injection.

even during the normal breeding season (fig. 2). This condition may be due to the absence of some essential psychic (or behavioristic) factor. Nest-building and mating are probably necessary prerequisites. That the late phase of greatest is suggested also by the fact that termination of egg laying is under the control of some part of the nervous system (7).

The lack of behavioristic stimulation is irrelevant in experimental groups receiving injections of gonadotropic hormones during or immediately before the normal breeding season. Injections of both beef pituitary extracts and pregnant mare serum have resulted in ovulation, eggs with normally colored shells being dropped in the cage or found in the uterus.

Domm (12) finds that the ovaries of chicks first injected with pituitary hebin 2 to 4 days after hatching show no follicular development. We obtained similar results with nestling passerine birds (unpublished data). On the other hand, Domm and Van Dyke (13) find that the ovaries of older females (21 to 47 days) enlarge, showing both follicular and medullary development. Riddle and Polhemus (14) injecting an extract of bovine anterior pituitary and a 'sex maturity' preparation from whole sheep pituitary into immature doves and pigeons (about 3 months old from beginning of embryonic development), report stimulation of the ovary. These authors observe, however, that the growth response of the ovary is about one-third or one-fourth that of the

testis. Since in the present investigation negative reactions were given by birds at least 3 months old (December series), it appears that the female sparrow remains in a refractory state longer than domesticated birds. It is to be noted that in the fall adult females resemble juvenile birds not only in ovarial size and endocrine inactivity, but also in their refractoriness to gonadotropic substances.

Whether the negative reactions given by some of the injected females in fall and winter is due to a refractoriness of the gonad or to the absence of essential supplementary activity by the bird's own pituitary, or even to the production of an inhibitory substance during the quiescent period is not cleared up by these experiments. It was thought that preliminary activation of the anterior lobe by light treatment might settle this problem. However, the results are not decisive. Experiments with hypophysectomized birds should prove more enlightening.

In general, it appears that the ovary of the bird is harder to stimulate than the testis. This fact, together with the low responsiveness noted in the fall and early winter help to explain the none too uniform effects of light treatment at these times. The low-grade responses of females treated with light in late winter and spring (when the ovary is responsive to gonadotropic substances) suggest that either the female anterior pituitary is less responsive to light than the male one, or that extensive follicular development is dependent upon some essential behavioristic stimulation thus far not obtained in the laboratory.

SUMMARY

Female sparrows were exposed to lengthened daily light periods during the fall, winter and spring. Exposure periods ranging from 21 to 75 days induce only a moderate degree of ovarial development. Female sparrows (including adults and juveniles) are partially refractory to injections of gonadotropic substances in September and October. Later, in the winter, fewer refractory cases are found and none after February. Ovulation and eggshell formation were observed in several cases. The ineffectiveness of additional daily light periods in the production of an extensive ovarial development indicates a low degree of responsiveness of the female pituitary to light. It is suspected that behavioristic factors, ordinarily involved in the nesting and mating activities, and which were not given free play in our experiments, are in some way very essential for the final hypophyseal stimulation and the growth of the ovaries to the breeding condition.

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SUBCLINICAL ADRENO-GENITAL SYNDROME

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Of all the clinical heterosexual anomalies, those associated with adreno-genital disease are by far the most common and perhaps the most significant. Inasmuch as the triad of the pituitary, the adrenals and the gonads are so intimately related in both the development and function of the reproductive system it is not surprising that similar disturbances of sex pattern and sex function may result from disease of any of these three glands. It is difficult to conceive of any significant pathology in any one of this triad of glands without influence on the other two, so that secondary effects may so color the original picture as to thoroughly confuse the clinician. That is chiefly why confusion has so often handicapped the differential diagnosis of Cushing's and the adreno-genital syndromes. Those differentiating features usually emphasized, such as the type of obesity or hirsutism, are not of sufficiently specific diagnostic importance.

Our chief concern in this paper is to point out certain hormonal differences between malignant and benign forms of the adreno-genital syndrome, emphasizing particularly the sex hormone aspects of the so-called subclinical syndrome. We believe that sex hormone assays are of diagnostic importance especially in differentiating between these two types of the disease.

The classical adreno-genital syndrome is characterized by varying degrees of virilism, and is usually ascribed to some demonstrable tumor or hyperplasia in the suprarenal cortex or ovaries. Gynecomastia may now be included in the category of adreno-genital syndrome (1, 2). The clinical aspects of these syndromes have been widely, although none too clearly, discussed. But the majority of patients, manifesting less striking features of sex reversal, reveal no clinically demonstrable tumor in either the adrenals or in the gonads. Obviously x-ray and surgical exploration will discover only the gross or macroscopic rather than any microscopic pathology which may be causing the disease. One is thus forced to seek other sources of clinical diagnostic aid.

Reports both here and abroad indicate that sex-hormone assays have been found extremely useful in this connection. Recent data of Koch (3), Callow (4), clearly show that the urinary androgens and estrogens are in far greater concentrations in the malignant type than in the benign types of the adreno-genital syndrome, thus segregating promptly the surgical from the non-surgical cases. This type of laboratory data is much more in evidence in the reports of tumor cases. The benign or subclinical forms have thus far received scant

attention; yet the incidence of this chronic benign syndrome is far greater. For example, of Cahill's 35 cases recently reported (5), only 6 had adrenocortical tumor. In the 30 cases observed by the senior author no tumors could be found by the usual clinical methods, including x-ray visualization of the adrenals.

Unfortunately, clinical investigation by means of sex-hormone assays have been handicapped because of frequent changes in the technical aspects. Recent modifications by Koch and his co-workers (6) have invalidated much of our previous data (7). The hormone assays incorporated in this report have been done in the past two months. Since using Koch's new technic we have recovered much higher androgenic concentrations than before. We realize that unless this newer, more satisfactory technic is universally adopted, male-hormone assays especially will have little or no value.

TABLE 1. NORMAL ANDROGEN-ESTROGEN RATIOS

Normal Males				Normal Females			
Androgens	Estrogens	A/E	Prolan	Androgens	Estrogens	A/E	Prolan
66	3	22.0	25	30	12	2.5	25
96	6	16.0	25	28	6	4.7	0
66	8	8.3	0	28	12	2.3	0
66	12	16.5	—	40	84	0.5	25
30	3	10.0	—	36	24	1.5	—
48	3	16.00	—	30	12	2.5	—
74	4	18.5	—	24	6	4.0	—
				20	24	0.8	0
				24	12	2.0	50
Average Values 64	5	15.3	—	29	21	2.3	—

OBSERVATION

Cahill's (5) recent clinical description fits fairly well the picture presented by our 30 patients observed in the past 5 years. This group consists of 28 females and 2 males with gynecomastia.

None of these patients manifested the dramatic reversal of sex to be seen with adreno-cortical tumor. But the clinical features occasionally are apt to be as bizarre as in the tumor cases. The chief complaints of the females were hirsutism, obesity and ovarian dysfunction, and this triad of symptoms was considered essential for the clinical diagnosis of the benign adreno-genital syndrome. Three of the females, however, were not obese, but the hirsutism and menstrual dysfunction were sufficiently advanced to justify their inclusion in this group. Feminine appearance was preserved in the majority; striking hypertrophy of the clitoris was rarely seen. The obesity and hirsutism is apt to be extreme in some, and moderate in the majority.

Menstrual function was grossly irregular, scanty or completely in abeyance for many months or years. Libido and fertility in the married women was sharply limited. In two there was familial hirsutism, and other familial endocrinopathies such as obesity, diabetes and goiter were much more common than in the general population. The psychological depression and anxiety states are difficult to analyze. How much of this psychic stress is to be ascribed to chaotic

hormonal chemistry and how much to cosmetic disfigurement is not easy to evaluate.

Some significant facts were revealed as to the age at onset of the disease: first, 20 of the females manifested symptoms at or soon after puberty, and 7 of the married women began to develop the syndrome soon after a pregnancy. All but 2 of the females developed their symptoms in early reproductive life. The disease is essentially one of long standing and of limited development.

TABLE 2. ANDROGEN-ESTROGEN RATIO IN BENIGN ADRENO-GENITAL SYNDROME IN FEMALES

Case No.	Androgens	Estrogens	A/E	Prolan
361	36	12	3.0	25
384	52	6	8.7	0
421	92	12	7.6	0
559	30	8	3.8	25
633	36	12	3.0	25
682	72	6	12.0	25
903	44	12	3.7	25
918	48	24	2.0	0
940	44	24	1.8	0
953	48	48	1.0	50
Average Values	51	16	4.7	—

The two males complained of gynecomastia; and the older man, aged 46 years, also complained of loss of libido and potency. The breast hypertrophy came on fairly abruptly at 44 and was associated with a perceptible decrease in size of testicles and penis. His beard required shaving as usual; his weight was moderately increased. The other was a young man of 19 who presented a well-developed adolescent right breast which had grown since the age of 16. His left testicle was atrophied and penis small—the general physique distinctly feminine.

The usual clinical laboratory data in all of the patients was essentially non-informative. There were minor discrepancies in the B.M.R., blood-sugar and

TABLE 3. ANDROGEN-ESTROGEN RATIO IN MALES WITH GYNECOMASTIA

Case No.	Androgens	Estrogens	A/E	Prolan
408	20	4	5.0	50
557	90	16	5.6	50

cholesterol determinations. One young woman has a chronic and severe diabetes mellitus and thus might be regarded as an example of a bearded female diabetic. The significance of her associated symptomatology is still speculative. Only one female was explored surgically without revealing any androgenic tumor in the adrenals or in the pelvis.

Pneumo-urography in 17 of the group by Cahill's (8) method of x-ray visualization of the adrenal failed to reveal any macroscopic tumor.

METHODS

Androgens were estimated according to the procedure of Gallagher, et al. (9). The values are expressed as international androgenic units per day. Estro-

gens were estimated by modifications of the existing methods on spayed rats. The values are expressed as gamma estrin per day. Prolan was estimated on immature mice. Vaginal canalization, plus a positive vaginal smear (appearance of cornified epithelial cells) were taken as a positive response. The values are expressed in mouse units per day. All the values were obtained on morning specimens pooled over periods varying from 7 to 21 days.

RESULTS

The sex-hormone data[1] shows essentially that the androgen-estrogen ratios seem concordant with the type of sex reversal observed clinically. The women excreted more androgens and occasionally much higher estrogens than do normal women, and likewise the men excreted abnormal quantities of estrogens, which may be in harmony with the breast hypertrophy. In other words, benign sex reversal states apparently are associated with a reversal of the normal sex-hormonal ratio or an imbalance of the various sex hormones. In many respects, our data agree with that of Koch and his co-workers, although our androgen-estrogen ratios tend to be higher than other published values.

However, the correlation of the clinical features and sex hormone assays is by no means uniform or regular. Much more quantitative data is urgently needed. That chaotic hypersecretion of sex hormones occurs in tumor cases is attested to by many workers (10,11,12) who have found enormous quantities of both androgens and estrogens in the blood and urine. We have not attempted to identify the various urinary androgens as yet. Perhaps a more meticulous chemistry may yield the bisexual hormone pregnane triol isolated by Marrian and Butler (13) in urine of women with adreno-genital syndrome.

DISCUSSION

Sex-hormone data in the benign types of the disease is still very meager. Enough, however, has been reported by Koch, Callow and others, as well as our present report, to point out the great differences in sex hormonal excretion in the benign and malignant cases.

Any attempt to evaluate possible mechanisms at play in the relatively benign adreno-genital syndrome would entail much speculative discussion far beyond the scope of this paper.[1] That the adreno-genital syndrome is usually associated with abnormal sex-hormone ratios seems fairly well established. At least enough is known to recognize the futility of surgical exploration of patients with virilism or gynecomastia, unless the evidence of x-rays and sex-hormone assays suggest gross tumor. Too many individuals have been needlessly subjected to this hazardous surgery without first having had such preliminary studies.

One must bear in mind also that in the subclinical syndrome one is very likely dealing with a widespread chain of polyglandular pathology (14) which must be of a microscopic rather than a macroscopic character. Therefore, for the differential diagnosis of the adreno-genital syndromes, sex hormone assays and x-ray studies hold much more promise than blind surgical exploration.

[1] A more comprehensive report and discussion will follow completion of this study.

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INSULIN-INSENSITIVITY: ITS POSSIBLE RELATION TO THE PITUITARY GLAND[1]

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The present knowledge of the glands of internal secretion suggests that there are extra-pancreatic endocrine factors which may influence carbohydrate tolerance and sensitivity to insulin. Sweeney (1) and others have shown the effect of varying amounts of carbohydrate, protein and fat on the glucose tolerance of normal individuals. It has been accepted generally that a high-carbohydrate, low-fat diet increases carbohydrate tolerance, whereas a diet low in carbohydrate and high in fat may so diminish carbohydrate tolerance as to produce a diabetic type of glucose tolerance curve in an otherwise normal individual. Recently, Himsworth (2, 3) reported that in normals the carbohydrate intake alone will determine both the glucose tolerance and the sensitivity to insulin. It follows that, the greater the response of the organism to insulin, the better will be the glucose tolerance. Improvement in carbohydrate tolerance in patients with diabetes mellitus has been observed following the use of the high carbohydrate regime.

The existence of 'insulin-fast' or 'insulin-resistant' diabetic patients has been reported by Lawrence (4), Root (5) and others. These patients can tolerate huge doses of insulin with no hypoglycemic reactions. Among the factors which are known to diminish the response to insulin are infection and ketosis, but cases have also been reported in which the cause for the resistance to insulin was not evident. The most recent of such reports is by Glen and Eaton (6) of a case of insulin-resistance following hysterectomy and ovariectomy. The blood serum of this patient, when injected into rabbits, prevented a fall in blood sugar following the administration of insulin. Despite this evidence of a substance 'antagonistic' to insulin, the source of the substance is speculative.

In 1936, Himsworth (7) reported a differentiation of diabetes mellitus into two groups by means of two blood-sugar curves. The first curve represented the response of the blood sugar to an ingested dose of glucose. The second curve represented the blood-sugar response to the same amount of glucose and the simultaneous intravenous injection of a standard dose of insulin. Those individuals in whom insulin suppressed the hyperglycemia that normally followed the ingestion of glucose were called 'insulin-sensitive,' whereas those in whom insulin did not suppress this hyperglycemia were

[1] This investigation was aided by a grant from the Milbank Foundation.

called 'insulin-insensitive.' In unpublished experiments, Himsworth found that in the insulin-sensitive group the curve following glucose and insulin could be altered by varying the amount of carbohydrate in the diet, as could the curve following glucose alone. When a low-carbohydrate diet was fed the sensitivity to insulin was impaired, and the curve following glucose and insulin approached the curve following glucose alone; whereas on a high-carbohydrate diet, the sensitivity to insulin was enhanced and the simultaneous injection of insulin suppressed the hyperglycemia. On the other hand, in the patient who had an insulin-insensitive type of curve on a diet moderately high in carbohydrate, the response to insulin could not be enhanced by further increase in the

TABLE 1. ACTUAL BLOOD SUGAR VALUES
Blood Sugar—Milligrams Per Cent

Patient	Experimental	Fast.	Fast.	Fast.	5 min.[1]	10 min.[1]	20 min.[1]	30 min.[1]	40 min.[1]	50 min.[1]	60 min.[1]	75 min.[1]	90 min.[1]	105 min.[1]	120 min.[1]
A (S.H.R.)	Gluc. 52 G. p.o.	95	94	96	100	114	151	171	180	161	152	98	98	86	72
Fig. 1	Gluc. 52 G. p.o. Ins. 8.6 u i.v.	93	90	88	90	92	88	93	92	92	86	80	78	77	—
B (G. F.)	Gluc. 56.7 G. p.o.	83	84	82	92	99	151	134	131	116	114	98	95	98	78
	Gluc. 56.7 G. p.o. Ins. 9.5 u i.v.	—	100	—	105	97	92	85	83	87	80	75	72	66	95
C (W. K.)	Gluc. 51.6 G. p.o.	90	92	91	98	114	111	104	127	147	160	164	150	123	93
	Gluc. 51.6 G. p.o. Ins. 8.6 u i.v.	92	90	89	90	92	80	73	85	93	95	98	90	—	—
D (S. R.)	Gluc. 51.6 G. p.o.	—	347	349	372	401	412	429	564	480	493	527	563	563	538
	Gluc. 51.6 G. p.o. Ins. 8.6 u i.v.	381	383	381	383	381	373	370	371	386	388	400	—	383	348
E (J. K.)	Gluc. 47.7 G. p.o.	240	237	244	256	276	338	404	432	474	499	524	515	507	—
Fig. 2	Gluc. 47.7 G. p.o. Ins. 8.0 u i.v.	228	232	232	244	243	220	213	200	210	223	261	276	251	—
F (F. S.)	Gluc. 56.1 G. p.o.	—	424	426	422	435	501	547	544	541	560	565	538	518	501
	Gluc. 56.1 G. p.o. Ins. 9.4 u i.v.	358	352	352	353	347	346	350	353	325	310	271	278	255	246
G (S. S.)	Gluc. 45.6 G. p.o.	247	245	240	234	251	289	299	303	339	318	—	354	353	353
Fig. 3	Gluc. 45.6 G. p.o. Ins. 7.6 u. i.v.	230	233	229	243	246	244	265	275	269	283	273	299	312	318
H (N. N.)	Gluc. 57.4 G. p.o.	215	219	219	215	233	272	299	295	305	297	299	265	259	255
Fig. 6	Gluc. 56.4 G. p.o. Ins. 9.4 u i.v.	235	237	233	238	253	263	289	295	299	293	278	274	253	246

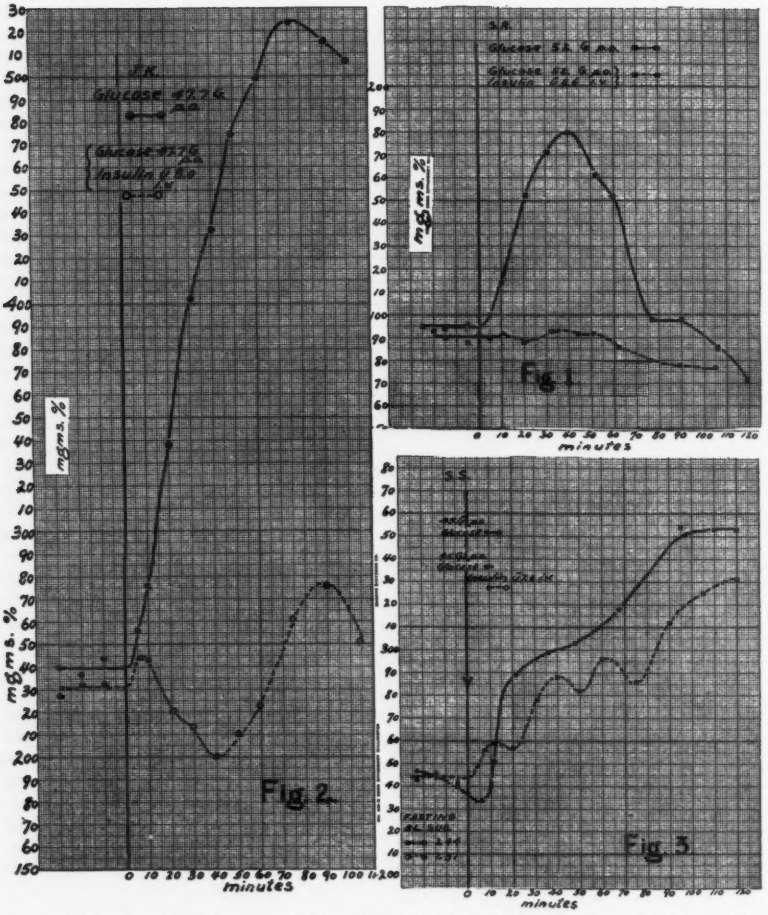
[1] Times are approximate. True values vary from 0 —3 minutes earlier or later, more often later.

carbohydrate intake. Thus, Himsworth's insulin-insensitive individual lacked the ability to respond normally to injected insulin.

The same classification but with different criteria was used by MacBryde (8) in 1936 to differentiate diabetics into an 'insulin-sensitive' and an 'insulin-resistant' group. The insulin-resistant individual, according to MacBryde, is the one who can tolerate a large overdose of insulin without developing hypoglycemic symptoms; whose blood sugar drops little after an injection of insulin; who metabolizes small amounts of sugar per unit of insulin as judged by the urinary excretion of sugar. The insulin-sensitive individual shows the reverse response. It was the insulin-resistant individuals who responded best to the high-carbohydrate regime. Their 'sensitivity' to insulin improved, as shown by the above tests. It is important that this insulin-resistant group of MacBryde should not be confused with the group described by Himsworth as insulin-insensitive. In Himsworth's group the high-carbohydrate diet had no

effect in altering the response to insulin. In other words, the 'insulin-resistant' individual of MacBryde constitutes an insulin-sensitive one whose sensitivity to insulin and whose carbohydrate tolerance have been impaired by a faulty dietary regime, but who responds well when the diet is altered.

There remains then a group of individuals who, for some reason, cannot



respond in the normal way to insulin, and this inability is not altered by a change in the diet. It is this group, in our opinion, to which the term 'insulin-insensitive' or 'insulin-resistant' should be restricted.

EXPERIMENTAL METHODS

In this study the blood-sugar response of 8 patients was observed by means of the blood-sugar curves described by Himsworth (7). The group consisted of 3 normal individuals, 3 patients with diabetes mellitus who were clinically

'insulin-sensitive,' and 2 patients with acromegaly and diabetes mellitus who demonstrated 'insulin-insensitivity.'

All blood-sugar curves were done in the fasting, resting state. The first curve was done after the oral administration of a standard dose of glucose; the second curve, after the oral ingestion of the same amount of glucose and the simultaneous intravenous injection of a standard dose of insulin. The dosage of glucose was 30 gm. per sq. m. of surface area; of insulin, 5 U per sq. m. of surface area. An interval of one day on the usual regime was allowed between the two curves. Blood samples were taken from capillary blood at approximately 5, then 10, and later at 15 minute intervals for two hours, and were analyzed by the Hagedorn-Jensen method (9). The diabetic patients were followed in the clinic and wards of the Third (New York University) Medical Division of Bellevue Hospital for 4 to 7 years, during which time they were on diets in which the carbohydrate varied from 180 to 300 gm., the protein 65 to 75 gm., and the fat was approximately 85 gm.

RESULTS

Figure 1 illustrates the typical response in the normal group (patients *A*, *B* and *C*). The diets of these individuals were unrestricted. They were all healthy, young adult white males. Following the oral ingestion of glucose the glucose tolerance was normal; the simultaneous injection of insulin completely suppressed the hyperglycemia of the first curve.

The 3 patients with diabetes mellitus (patients *D*, *E* and *F*) were males who had had diabetes for from 7 to 14 years. At the time of this study they were all well-controlled on diets of carbohydrate 200 to 250 gm., protein 65 gm. and fat 85 gm., and insulin 60 to 75 U daily. Each had been in ketosis at least once, and there was a marked tendency to hypoglycemia when deviation from the approved routine occurred. Figure 2 represents the type of curves obtained in these patients. The glucose tolerance curves were markedly abnormal in all these patients and, in all, the simultaneous intravenous injection of insulin completely suppressed this hyperglycemia.

Figure 3 illustrates the curves obtained in a 35-year-old white female (patient *G*) with classical signs (fig. 4) and symptoms of acromegaly of 5 years' duration and diabetes mellitus of 4½ years' duration (10). Because of persistent glycosuria, her insulin dosage was raised gradually to 200 U daily. During a period of hospitalization the urine was consistently sugar-free and there were no hypoglycemic reactions on a diet of carbohydrate 180 gm., protein 65 gm. and fat 85 gm., and insulin 200 U daily. The dosage of insulin was decreased to determine her minimum requirement, and 2 weeks later she remained sugar-free on the same diet and no insulin. During this period the curves shown in figure 3 were obtained. The glycosuria returned and persisted in spite of resuming insulin and finally returning to the original dosage. Blood-sugar curves were done again during the glycosuric period and were essentially the same as those in figure 3. In spite of prolonged and marked glycosuria, ketonuria was never observed in this patient. From figure 3 it is apparent that

the administration of insulin had little effect in suppressing the hyperglycemia that followed glucose alone.

The blood-sugar curves of the second patient with acromegaly and diabetes (patient *H*) are shown in figure 5. This patient was a 49-year-old white male, who was first observed in 1930 when he entered Bellevue Hospital because of xanthoma diabeticorum (11). At that time the features of acromegaly were well advanced. On that admission the urine showed marked glycosuria and ketonuria, which responded well to diet and insulin therapy. For the last 7 years he has been observed in the clinic and maintained on a diet of carbohydrate 175, protein 65 and fat 85 gm., with insulin 50 to 90 U daily. Glycosuria has been present at times over this period, but there has been no ketonuria. The blood-sugar curves obtained in this patient are similar to those of patient *G*, in showing that the insulin administered had little effect in determining the type of blood-sugar curve; the curve following glucose and insulin differed little from the curve after glucose alone. In this instance, however, the curves are of a different type from those in figure 3. There is an almost normally-shaped glucose tolerance curve, in spite of a high fasting-blood-sugar level and evidence (the glucose and insulin curve) to show that the insulin injected had little effect in depressing the blood sugar.

Fig. 4. Patient *G*. Tumor of the pituitary gland with acromegaly and diabetes mellitus.

DISCUSSION

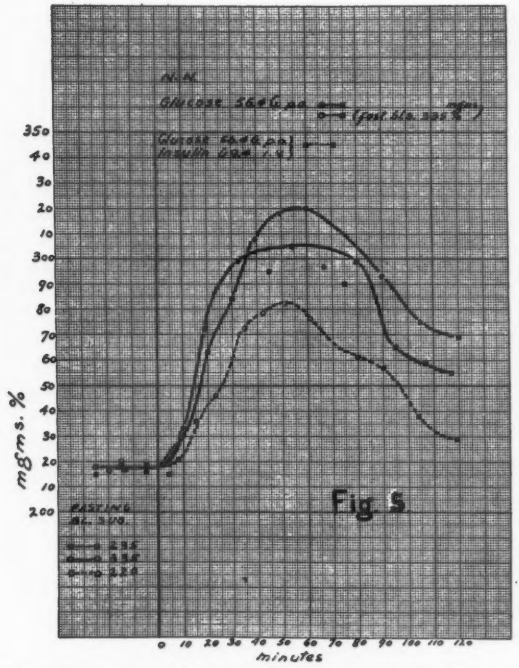
The blood-sugar curves obtained in the 'insulin-sensitive' group of diabetics are similar in contour to those of normals, but occur at higher blood-sugar levels. In both groups there is no significant rise from the fasting blood-sugar level after the simultaneous administration of glucose and insulin. The similarity in the resulting curves lends support to the view that in the 'insulin-sensitive' diabetic, the diabetes is due to a lack of insulin. When insulin is supplied to these individuals, they react to it in the normal manner, i.e., by a suppression of the hyperglycemia.

The response of the patients with acromegaly is different. The normal action of insulin in depressing the blood-sugar level is diminished. That these patients are 'insulin-insensitive' is apparent from both the curves obtained and the clinical course described (patient *G*).

The rôle of the pituitary gland in carbohydrate metabolism has been clarified by the work of Houssay (12) and Long (13) who demonstrated that hypophysectomy improved the diabetes produced by depancreatization. The hyperglycemic effect of pituitary extracts administered parenterally is well

known (14). In view of these facts, and as tumors of the pituitary gland were demonstrated by x-ray in both patients with acromegaly (and by post-mortem examination in one), it is likely that the hyperactivity of the pituitary gland is an etiologic factor in the 'insulin-insensitivity' in these individuals.

All evidence points to the fact that the 'insulin-sensitive' type of diabetes is due to a lack of insulin. In the 'insulin-insensitive' type of diabetes it seems more probable that some factor interferes with the normal action of insulin. It is possible, therefore, that the syndrome of diabetes mellitus may be present in an individual whose pancreas secretes a normal amount of insulin.



The blood-sugar curves shown in figure 5 are difficult to explain. In spite of high fasting-blood-sugar levels and evidence that insulin had little effect in determining the type of curve obtained, they are almost normally-shaped glucose tolerance curves. These curves resemble the 'normal' glucose tolerance curves obtained by Soskin et al (15) in depancreatized dogs receiving a continuous infusion of glucose and insulin to maintain a constant blood-sugar level. In this patient, however, the insulin injected did not alter the curves significantly.

DeWesselow and Griffiths (16) used a modification of the simultaneous glucose-and-insulin curve described by Himsworth in studying a group of diabetics. They report no correlation between the type of curve obtained and certain clinical phenomena. It is our opinion that this type of blood-sugar curve is of little value in determining the 'type of diabetes present, unless (1)

it is performed after the individual has been on a moderate or high carbohydrate diet in order to insure maximum sensitivity to insulin, and (2) it is compared with a curve following glucose alone to note the effect of insulin in suppressing the postglucose hyperglycemia. In the group of patients in whom deWesselow and Griffiths noted the effect of diet on this curve, each patient showed improvement (i.e., a depression) of the glucose-and-insulin curve after the period on a high carbohydrate diet. Because of this beneficial result of dietary treatment this group could be classified as 'insulin-sensitive' diabetics, and is comparable to the group described by MacBryde as 'insulin-resistant' described above. The simultaneous glucose-and-insulin curve is of more value in studying the mechanism of various disorders of carbohydrate metabolism than as a routine diagnostic procedure.

SUMMARY

Three groups of patients were studied by means of the glucose tolerance and simultaneous glucose-and-insulin blood-sugar curves. The 'insulin-sensitive' diabetics resemble normal individuals in showing a suppression of the postglucose hyperglycemia in the glucose-and-insulin curves. In two patients with acromegaly and diabetes the simultaneous intravenous injection of insulin had little effect in suppressing the postglucose hyperglycemia. It is suggested that the tumor of the pituitary gland demonstrated in these patients is an etiologic factor in the 'insulin-insensitivity' exhibited by these patients.

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JUVENILE ADIPOSOGENITAL DYSTROPHY. NEUROLOGIC AND PSYCHOPATHOLOGIC ASPECTS. RESULTS OF ORGANOTHERAPY AND PSYCHOTHERAPY[1]

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The syndrome of adiposogenital dystrophy was established first by Babinski (2), then by Fröhlich (3). Their respective cases presented obesity and genital atrophy, together with signs of an expanding lesion in the region of the pituitary fossa. Later the term came to be applied to the syndrome in general, regardless of etiology. This paper is based on the study of 16 boys, varying in ages from 4 to 14 years. None of the patients showed signs of intracranial expanding lesion, such as tumor or hydrocephalus; none was of moronic or of lower intelligence; none gave a history of encephalitis. The neurologic and psychologic problems studied would be completely altered by the presence of such grave lesions.

The patients presented most of the following endocrine symptoms: adiposity, consisting of generalized fat deposits, accentuated about the lower and upper girdle, or of fat deposits only about the upper and lower girdle; genital anomalies; small penis with redundancy of the skin; undersized, often unilaterally or bilaterally undescended or maldescended testes; skeletal anomalies, the lower measurement greater than the upper span of outstretched arms frequently greater than the height; genu valgum; pes planus; anomalies of hair distribution; absence of pubic and axillary hair; low hair line on the forehead and temples almost meeting the eyebrow laterally; occasionally hirsutism of the extremities; fine, though often dry, skin, particularly on the dorsum of the hand and on the torso; tendency to low basal metabolic rate,[2] ranging mostly from —20 to —10 (if the child was too anxious, the test was discarded). The sugar, cholesterol and uric acid values in the blood were within normal ranges.

This syndrome of adiposogenital dystrophy of the type described in adolescence is a curable condition.

METHODS

In addition to a routine neurologic examination, a special study was made of 3 so-called postural reflexes as standardized by Hoff and Schilder (4) on normal adults. See also Magnus (5), Mittelmann (1b), Fisher and Wodak

[1] Read at a meeting of the New York Neurological Society, October 1935 (1).
[2] For calculating the basal metabolism under 10 years of age the Benedict-Talbot Normals (Carnegie Institute of Washington, 1921) were used; above 10 years the Harris-Benedict Normals (1919). For the test, either the open or the closed circuit method was used, sometimes both on the same patient. The results obtained with the two methods tallied closely.

(6), Goldstein and Riese (7), Zingerle (8). *a),* In the reaction of divergence the patient holds the arms outstretched at shoulder level, with eyes shut; the arms go slowly into abduction; *b),* In the neck-arm reflex the patient is asked to hold both arms at shoulder height; the examiner rotates the patient's head first to one side, then to the other; the arms move in the direction of the chin, the chin-arm rising and the occiput-arm sinking; *c),* In the reaction of maintained muscle tone the subject holds one arm at shoulder height and the other arm raised 80° above the horizontal, with the eyes shut, for 20 seconds; he is then asked to lower the raised arm to the level of the horizontal arm. The arm which was elevated stops at a level above the horizontal. Schilder (9a) remarked that in adolescence all of the reactions tend to be increased. For a standard of comparison 20 adolescent boys were examined in this study.

The method of psychopathologic survey was the one usually practiced in mental hygiene clinics: psychiatric history, observation of the patients' behavior and continued contact with the patient and the family.

General Account of Neurologic and Psychopathologic Findings

All of the children presented generalized muscular hypotonia, but as a rule the tendon reflexes were not diminished. Some children showed a peculiar clumsiness and difficulty in the execution of skilled movements, at times with otherwise normal neurologic findings. Standing and walking had not been retarded in infancy, but the children had a tendency to stumble and fall. Later, they could not compete well in sports, and their lack of skill and clumsiness was noticed by their classmates and parents. Another occasional finding was a fine tremor of the intention-tension type, absent at rest and appearing almost in infancy; it was noticed by the patient's parents and classmates. Some children showed an inequality of the associated arm movements on both sides. Another finding was the presence of marked coarse involuntary movements resembling choreo-athetoid movements, particularly in the outstretched arms, but also in legs and neck.

The postural reflexes, in addition to a frequent increase over the adult norm, showed certain deviations. In some cases the outstretched arms were adducted (reaction of convergence, Schilder) in the shoulder joint, at times accompanied by flexion in the elbow joint. In other cases, when the head was rotated the chin-arm, instead of being abducted and elevated, was adducted and lowered (paradoxical reaction, Schilder). Other patients, when given the command to lower the elevated arm to the level of the other horizontal arm, lowered the former to the exact level of the latter or below it (lack of maintaind muscle tone).

The 20 normal boys examined showed an increase in all postural reflexes as compared with adults. Some of the children showed reaction of convergence and some of them a paradoxical reaction, but less frequently than was found in the adiposogenital group. Among the 20 normal children, reaction of convergence was found in 2; among the 16 children with adiposogenital dystrophy, reaction of convergence was present in 5 and paradoxical reactions in 5; four showed lack of maintained muscle tone in one or both arms. None of the

normal children showed this phenomenon. Involuntary muscle contractions resembling choreo-athetoid movements were described in normal adolescents by Homburger (10); however, some adiposogenital dystrophy cases showed this phenomenon in greater intensity. One child in the adiposogenital group showed spasmodic torticollis.

A general survey of the overt psychopathologic manifestations in the adiposogenital dystrophy group showed marked submissiveness, overdependent attitudes; depressions; weeping spells; infantile phobias (fear of animals, etc.); habit spasms, and compulsive manifestations. Of these, particularly the disturbances of attitude and behavior (over-dependent and over-aggressive) and the disturbance of mood (depression) were found to be related to the syndrome.

The psycho-pathogenic factors involved can be grouped into two classes: *a)*. Direct pathophysiologic influences on psychic functions; *b)*, Reactions of the total personality to circumscribed deficiencies. The lowered metabolism, indicative of a poverty of drive has a direct pathophysiologic influence on the psychologic functions; but low metabolic rate (in one patient —23) was compatible with normal competitive spirit and stamina. Other times, marked overdependence and passivity were found with normal basal metabolism. Another pathophysiologic factor was an excess drive for certain mouth functions. All of the boys had good appetities, rarely presenting feeding difficulties after the first year of life. A history of nail-biting, eating sand, chewing pencils, etc., was common; but late thumb-sucking was infrequent.

The patient may come to accept his deficiency of drive with his total personality and effect his whole adaptation under its influence. The neurologic signs and endocrine symptoms, affecting the appearance of the body, influence psychopathology in terms of the reaction of the total personality to circumscribed deficiencies. The patients are impeded in competitive games and ridiculed because of their obesity, and their inability to execute skilled movements well (tremor, clumsiness). These deficiencies represent a blow to the children's self-esteem (narcissistic injuries), to which they react with a feeling of inadequacy (9c, 11, 12). They may give up the attempt to be other children's equals in physical prowess (avoidance of the painful situation), become overdependent on their mother, shy and anxious, and play with girls in preference to boys (reaching new shelter and substitutive gratifications). This type of adaptation may cause parental disapproval because the boy does not conform to their masculine ideals, or his behavior interferes with their convenience.

Other patients, however, sometimes after an initial period of over-dependence respond to the factors enumerated with hostility and aggression (12). The over-aggressive behavior is usually limited to certain situations or persons: to schoolmates, to the mother and siblings, etc.; and some features of overdependence are retained (wanting to be in the mother's presence, weeping spells, anxieties). This reaction represents an attack on those (schoolmates) who actually injured the patient's self-esteem, or the patient may be projecting this injury on to persons in his environment, and in that way blame them for his own somatic deficiencies. The reaction, moreover, is a renewed attempt at

TABLE 1

Patient	Age yrs.	No. yr. observ.	Endocrine symptoms	Neurological symptoms	Psychopathology			Follutein used	Effect of follu.	Therapeutic Results		
					Over-dependent	Over-aggressive	Other symptoms			Endocrine	Neurol.	Psychopathic
S.S.	10½	6	Small genitals, skelet. disprop. obesity, B.M.R. −14	Hypotonia, tremor converg., unequal. assoc. arm movm. paradox. neck-arm reflex.	+	+	Phobias, habit spasm	0	—	Spontaneous correction with thyroid	Unchanged except hypotonia	Corrected
P.F.	11½	4	Small genit. undescend. testes, obese.	Hypotonia, choreoath et, converg. lack of maintained muscle tone.	+++	—	Some anxiety	+	Testes descend. genit. develop.	Genit. corrected, obese. persists	Unchanged	Corrected
B.M.	9	1	Small movable testes B.M.R. −10, skeletal disprop. obesity.	Hypotonia, choreoath et, converg.	+++	+	Slowness, habit spasm	+	good	Partial correction		Corrected
J.B.	11½	2	Undesc. testes, skelet. disprop., small genit. obese.	Hypotonia, lack of maintained muscle tone.	++	0		+	Testes descend.	Corrected genit. obese. persists		Improved
P.W.	12	5	Small genit., skelet. disprop. obesity B.M.R. −15	Muscular hypotonia.		Hyperkinetic +++	Lying, stealing	0	—	Spontaneous correct. with thyroid		Corrected
A.B.	14	2	Small genit., skelet. disprop. obesity B.M.R. −15	Converg. swings one arm more.		Hyperkinetic		+	—	Corrected	Unchanged	Unchanged not serious
A.M.	14	1½	Small genit. undesc. testes, skeletal disprop. obese, B.M.R. −29	Hypotonia.		+	Depression	+	Testes, descended, genit. develop.	Genit. correct. obese per.	Unchanged	Corrected

TABLE 1. (Continued)

Patient	Age yrs.	No. yr. observ.	Endocrine symptoms	Neurological symptoms	Psychopathology			Follutein used	Effect of follu.	Therapeutic Results		
					Over-dependent	Over-aggressive	Other symptoms			Endocrine	Neurol.	Psychopathic
J.G.	9½	2	Girdle adiposity, skelet. disprop. small genitals.	Clumsiness, lack of maintained muscle tone.	+	++	Severe anxiety	0	—	Unchanged	Unchanged	Improved
J.S.	4½	Seen twice	Obese, small genit.	Spasmodic torticollis.	0	+	—	0	—	Not treated		
J.K.	12	1	Obese, small genit. movable testes, skelet. disprop.	Unequ. assoc. arm movm., converg., no maint. muscle tone.	+	++	Severe compulsion and tic	0		Unchanged		Corr. by psycho-analysis
R.F.	13	2½	Obese, small genit. undesc. testes, skeletal disprop.	Hypotonia, choreoath et, converg.		+	Lying, stealing	+	Good	Genit. corr. obese persists	Unchanged	Corrected
L.C.	19	1 mo.	Obesity.	0	+	+	Severe anx.	0		Obese per. genit.	Unchanged	Improved
M.W.	12	1	Girdle adiposity small genit., disprop. skeletal.	Hypotonia.	0	0	Slight depression	+	Good	Corrected	Unchanged	Corrected
P.R.	4	3 mo.	Obese, undesc. testes.	Hypotonia.	0	0	0	+	Testes desc.	Corrected	Unchanged	Corrected
S.G.	8	6 mo.	Obese, undesc. testes.	0	0	0	0	+	Test. had been oper.			Corrected
S.M.	15	3	Girdle adiposity small genit., skelet. disprop.	0	0	0	Mild anxiety	+	Good	Corrected		Not treated
Total 16				Positive Total 13	+Total 7	Total +10	+Total 11	Total +10	Total 9	Favorable 12	Unchanged 9	Favorable 14

mastering difficulties and may offer substitutive gratifications through pleasure in cruelty.

The patient's reaction to the motility disturbance and poverty of drive has still another aspect. He experiences discrepancies between his aims and his abilities to execute them (13). The resulting discomfort leads to developments similar to the narcissistic injury (over-submissive, or destructive adaptation).

The secondary effects of the excess 'oral drive' may be complex: eating sand, chewing pencils, etc., arouse the opposition of the parents, leading to fear of punishment and of loss of love in the patient. But the patient may not succeed with restraining and repressing his desires. Similar difficulties may arise if he attempts to diet. His conflicts with himself and with his environment, accompanied by a feeling of worthlessness, of not being loved by those on whom he is dependent, lead to an increase in his helplessness, anxiety and hostility (14). These factors in turn manifest themselves in further acts of hostility, in depression, anxiety states and conversion hysterical symptoms.

The relative predominance of over-submissive or over-aggressive reaction type in a given patient is correlated partly with the age at which the various symptoms became a serious problem, and partly with the child's relationship with his parents and siblings.

The motility disturbance, manifest as a rule before the age of 5 years, tends to make the child over-dependent; so does early presence of obesity; whereas its later appearance is prone to make him over-aggressive. The parents may notice the child's genital anomalies very early or late, but it is difficult to determine at what age the children themselves became aware of this symptom. They often state that this did not occur before 8 or 9 or even 12 years of age. In many such cases there is evidence that an unconscious knowledge of the deficiency has been present before. These children, as a rule, do not urinate in the company of other boys and do not compare their genitals. In every case, however, when the patient is inescapably confronted with the fact of the smallness of his genitals (narcissistic injury) at adolescence, e.g., in the shower-room, etc., the reaction is a definite feeling of inadequacy, depression and hostility. An early knowledge of this tends to make the patient over-dependent.

An over-protective mother will push the patient in the direction of submissiveness and over-dependence. A domineering parent will do the same in the patient's earlier years, with, however, a change to defiance in adolescence. Punishment and rejection of the child by the parents because of his deficiencies will lead to developments similar to those mentioned as secondary results on the excess 'oral drive': feeling of worthlessness, of helplessness, anxiety, hostility, depression, and hysterical manifestations. All cases with psychopathologic manifestations finally show an admixture of the various types of components enumerated, but the relative importance of these components will vary with each case.

The hyperkinetic behavior disturbance can be differentiated from the over-aggressive type. Common to both is defiance, disobedience and often destructiveness. In the hyperkinetic disturbance, the problem of supervision and

management start almost in infancy and it manifests itself in all situations: at home and in school. During the interview the patient's hands are always busy with some object on the examiner's table, despite repeated requests to the contrary and with a few minutes' interruption at best. Despite good contact between the physician and patient there is little immediate therapeutic response of even temporary (few hours) nature. The basic disturbance in the hyperkinetic behavior seems to be a constant motor drive (or the absence of check on it), to which the patient must yield. As a type of pathogenic factor it should be listed with the low metabolic rate and the marked 'oral drive.' Its effects become combined with the reactions of the total personality to it, acceptance or attempts at control—and with any of the other factors previously discussed. The hypermotility in this series of patients was relatively rare and was never as marked as it is observable in postencephalitic children.

Some cases of juvenile adiposogenital dystrophy do not show gross psychopatholigic signs, despite marked endocrine symptoms. A careful study of such cases gives indications of similar pathogenic reactions as found in the severe cases. It is often unclear how such cases escape more marked psychopathologic symptoms.

THERAPY

The genital signs, low B.M.R. and obesity in many cases disappear about or after puberty without any treatment. The skeletal disproportions may undergo partial correction. The cure, however, can be hastened or be brought about, if necessary, by adequate treatment. The genital under-development responds to injections of pregnancy urine extract. The initial dose injected was 50 R.U. of follutein (Squibb), raised by the same amount with each subsequent injection to avoid local and general reaction. The dose was worked up to 400 U 5 times a week, and was continued until definite signs of genital development were present. The descent of the testes usually occurred within 10 to 14 days after the beginning of the injections. Pubic hair growth appeared within 1½ to 3 months, increase in the size of the testes about the same time, and increase in the size of the penis soon thereafter. If the metabolic rate was low, thyroid was administered in sufficient amounts to raise it to —5. Before the P.U. extract was available on the market, some patients in this series received thyroid substance only. A number of them developed genitally within the first year or two after the medication was initiated. In the absence of a large enough control series it is difficult to decide whether the thyroid substance hastened the development.

Obesity sometimes disappeared spontaneously with the genital development and with the correction of metabolic rate, whereas in other cases it required dieting.

The diet given in this study contained 750 cal. daily with 1 gm. protein per kg. body weight and provisions for vitamins. Dieting for the patients often presents serious difficulties because they find it hard to curb their appetites. The patient's attachment to the therapist, however, is often strong enough to help him overcome this difficulty. If the struggle is too hard, and disobeying

the dietary rules leads to clashes between the patient and parents, it is best to abandon the attempted weight reduction, at least temporarily.

In addition to the correction of the physiologic defect, the psychotherapy consisted of giving the patient and his parents as much understanding as possible of his special difficulties and showing him adequate sympathy. The parents, particularly the mothers, often show marked emotional reactions toward the patient's symptoms. The reactions are bound up with their own frustrations and conflicts and are difficult to correct; but unless a measure of success is achieved in this, the child will retain much of his psychological abnormalities.

The motility difficulties tend to persist after the correction of the endocrine symptoms. Even in such cases, the patient's reaction to them can be improved by psychotherapy. Cases with predominantly aggressive and hostile features of behavior anomaly, but with a good environment, show the quickest and best therapeutic response. Their attitude is relatively close to the 'healthy' aggression of physical exercise and contact with playmates. Over-dependent patients with understanding and considerate parents are particularly difficult to correct. There is too little pressure to force a change. Hyperkinetic behavior anomaly persists after the endocrine symptoms and is particularly difficult to treat. The patient is apt to commit petty crimes and land in jail or in a correctional institute. But the outlook is not helpless.

CASE REPORTS

Case 1 illustrates the complexity of psychopathogenic factors. *S.S.* had been obese (fig. 1A) since infancy. He sat, walked and talked at about the usual time, learned sphincteric control early, and was not a thumb-sucker, but stumbled and fell frequently, up to about the age of 6 years. The trembling of his hands and head was noticed for the first time when he wrote in school. It showed no remissions, but was more marked when he was excited. He spilled liquids from the spoon. When he was 6 years, the smallness of both testes and the mobility of one was noticed by a physician. When first seen at 10½ years his height was 55 in.; span of arms 56 in.; lower measurements 29 in., and he had genu valgum, large central incisors and generalized obesity accentuated over the upper and lower girdle. He weighed 108 lbs. (48 per cent above the median);[3] the contour of his body was feminine; his shoulder width was 13½ in., his hip width 12½ in. His penis was small with a circular groove at its root and redundancy of its skin; the testes were small and soft in the scrotal sack, with a deep central groove in the scrotum; the skin was fine over the body but of definitely darker hue than the skin of other members of the family; there were two darkly pigmented spots on the torso of the size of a silver dollar and of irregular shape; the hair-line was low, with an excess of lanugo hair on both forearms; there was excessive perspiration of the palms of both hands and of both arm-pits. His B.M.R. was —14. The neurologic examination disclosed muscular hypotonia (double joints); stronger associated arm movements on the right than on the left; medium fine rapid tremor; occasionally perioral tremor; tremulous voice, speech somewhat resembling the staccato type; postural reflexes; reaction of convergence, being due partly to adduction in the shoulder and partly to flexion in the elbow joints, and paradoxical neck-arm response on the left.

[3] Calculated from the Wood-Baldwin tables.

In the third year of observation (age 13 years), the patient developed epiphysitis of both tibial tuberosities (Osgood-Schlatter's disease). This disappeared in one year.

The patient's mother, the dominant member of the family, was strict, anxious and overprotective. She inspected the patient's bowel movements daily, and watched his 'trembling' and talked about it. Up to 2 years before coming to the clinic she had wanted him within earshot even on the rare occasions when he wanted to go with other boys. The patient's father was an unassuming person; the patient's sister, 3 years the patient's junior, was a well-behaved healthy girl, her mother's favorite.

About the age of 5 or 6 years, the patient found himself unable to compete effectively in sports. After a period of distress he gave up further attempts, and instead played with his sister, read books and progressed well in school. His teacher was considerate in regard to his hand tremor. He was peaceful at home and did not fight with his classmates even when they called him 'fat and sissy.' He has had a fear of insects and of darkness as far back as he could remember. These symptoms stopped at the age of 9 years, coinciding with the change in the patient's general adaptation. Following a sty on his eyelid, about the age of 9, he began to blink for recurrent periods of time.

At about the age of 9 years he stopped playing with his sister, teased and hit her, became disobedient and abusive with his mother, began to play with his classmates and beat them up if they 'called him names.' In addition to his previous nail-biting, he now chewed on his ties and on the collar of his shirt. Several factors combined in producing this turn in the patient's adaptation. The motive of sibling rivalry was one; his sister entered school and became the object of added interest for the mother. Further, his classmates, some of them nearing puberty, became increasingly rough and teased him about his obesity, his playing with his sister, etc. (blow to self-esteem, narcissistic injury). The under-development of his genitals had a marked and complex effect on the patient. The mother had examined the patient's scrotum and testes at the daily bath since he was 6, and would remark: "Dr. X said that one was too small, but it is not so." At the age of 9 years the patient refused to be bathed by his mother. However, he told the examiner that he first knew of his genital condition at the age of 9 years, after a visit to another clinic, thus covering up the daily experiences of 3 years. Because of the proved amnesia, it could not be determined to what extent the renewed painfulness of this knowledge (e.g., due to remarks of boys in the shower-room) played a primary rôle in changing this patient's adaptation. He now felt bitter and inferior about this symptom. The knowledge of, and daily experiences connected with, his small genitals (implying passive child-like rôle, deficiency, 'castration,' 'masturbation' by the mother) had pushed the patient in the direction of a submissive, over-dependent attitude, until this knowledge became too painful (classmates' remarks) whence it became the source of over-aggressiveness. His reaction to his tremor underwent a similar change. This symptom (blow to self-esteem, inability to master a function, over-solicitude of the mother, etc.) had first pushed him in the direction of a submissive adaptation, and later in the direction of over-aggressiveness, the chief objects of which were his sister and mother (rivals and aggressors, also representatives of his deficiencies). He treated his father with contempt. ("You are as I used to be.") His work and conduct in school remained good throughout.

Desiccated thyroid was administered up to 3 gr. daily, which raised the B.M.R. to +5 for about one year, when signs of genital maturity appeared. At 15 years,

the size of penis and the amount of hair on the body and face were above the average for his age, and he was shaving twice a week. He had lost his obesity without dieting. His height was 67 in.; his weight 130 lb. (7 per cent above the median). His body showed a somewhat feminine contour and the neurologic symptoms, except for the muscular hypotonia, remained unchanged.

There was not much improvement in the patient's behavior in the first 2 years of observation. Intensive psychotherapy was then started. For about 6 months the patient and his mother were seen at weekly interviews, lasting about one half hour each. Later, they were seen once or twice a month. With adequate sympathy the causes of his reactions were explained and illustrated to the patient, on the basis of daily incidents which he or his mother related to the examiner. He was encouraged to be self-reliant, but it was pointed out to him that his reactions overshot the mark. The mother was persuaded to give the boy more liberty, and to stop watching over him. The patient's behavior improved. The symptom of blinking with the eyes returned on a number of occasions; it served the purpose of both gaining the mother's attention (dependence) and of defying her (hostility). Because of his persistent tremor, eating with others remained a problem for the patient. After the mother stopped watching it, he was willing to eat in the circle of his family, but not in the presence of visitors. If she tried to persuade him to join, periods of abusiveness returned. A situation like this meant a renewal of all previous injuries to the patient. He could deal with such a threat only by avoiding the situation. He was observed for 6 years.

The neurologic symptoms with the exception of the hypotonia remained unchanged.

Cases with Over-dependence Predominating

Case 2. P.F., when first seen at 11½ years of age, showed obesity and undescended right testis, both conditions having been present since infancy. His height was 64 in.; lower measurement 32 in.; span of arms 64 in.; he had generalized obesity accentuated over upper and lower girdle. He weighed 154 lb. (29 per cent above the median); his B.M.R. was —6; his penis was small with a groove at its root and redundancy of its skin; his left testis was palpable but soft, his right testis was undescended; the hair-line of his scalp was low; he had a high-pitched voice.

The neurologic examination disclosed generalized muscular hypotonia; involuntary movements of the shoulders, the head, but particularly of the outstretched arms, lack of maintained muscle tone, and reaction of convergence. The patient has been inactive in sports and shy all his life, and has always wanted to be with his mother. He was the older of two siblings. The mother was the dominant member of the household but not too strict or over-solicitous. He showed 94 ideational, 81 motor intelligence.

The patient received 3 gr. of thyroid daily for 2 years, and injections of follutein for 4 months. The genital symptoms were corrected, but not the obesity and there was no change in the patient's general adaptation. He came for psychotherapeutic treatment irregularly and did not follow his diet. The mother's attitude was understanding and lenient. There was nothing to force him to change. He was followed for 4 years.

Case 3. B.M. had been obese since infancy; the smallness of his genitals was noticed when he was 5 years of age. When first seen at the age of 9 years his height was 52¾ in.; span of arms 53 in.; lower measurements 28 in. He displayed genu valgum. He weighed 79 lb. (17 per cent above the median). His penis was

small, with redundancy of skin and a deep groove at its root. His testes were small and slid up into the inguinal canal. The hair-line of his scalp was low; his voice high-pitched. His B.M.R. was —10.

The neurologic examination disclosed slight reaction of convergence and choreo-athetoid movements in the outstretched arms. His mother was very domineering, over-solicitous, suffering from depression and anxieties: "I am afraid he won't grow up to be a man. If he turns out to be a homosexual I'll kill myself." She always argued with the examiner. The patient, an only child, always wanted to be with her. He only played with girls younger than himself.

The endocrine symptoms were corrected by the administration of follutein, thyroid (2 gr. daily) and dieting.

The psychotherapy administered to both the patient and the mother was successful. The discomfort caused by the mother's attitude made the patient responsive to therapeutic efforts. He was followed for 1 year.

The patient's 'girlish' behavior sprang from 3 sources: direct psychologic effect of the metabolic disturbance; reaction of total personality to that and other deficiencies and the response to a domineering and over-protective mother.

Case 4. J.B. had been obese since infancy. When first seen at 11½ years of age, his height was 60 in.; the span of his arms 61½ in.; lower measurement, 32 in. He weighed 121 lb. (24 per cent above the median). His penis was small; his testes bilaterally undescended. The hair line of his scalp was low. The neurologic examination disclosed generalized muscular hypotonia and lack of maintained muscle tone.

His parents were over-solicitous and saw nothing wrong in the patient's behavior. He was an only child. He was shy, obedient to his parents and never fought with other children. He was so much afraid of the basal metabolism test that it could not be taken adequately.

Both testes descended 10 days after follutein and thyroid medication was started. No psychotherapy was given. There was some increase in the patient's aggressiveness in the course of 2 years following the treatment, but his adaptation remained predominantly over-dependent.

Hyperkinetic Cases

Case 5. P.W. weighed 9½ lb. at birth, then lost weight because of intercurrent infections, but has been obese from the age of 3 years. His genitals were noticeably small in infancy. When first seen at 12 years of age, his height was 63 in.; the span of his arms 63½ in.; his lower measurements 33½ in.; his shoulder width 14 in.; his intertrochanteric distance 13 in. He displayed genu valgum and long tapering fingers. Small penis with redundancy of the skin and a groove at the root; small testes; obesity accentuated about the upper and lower girdle. He weighed 145 lb. (28 per cent above the median). His B.M.R. was —14.

The neurologic examination disclosed generalized muscular hypotonia. He had been restless as an infant, and over-active and destructive since he was able to crawl around. He was a disciplinary problem in school. He was abusive to his mother, exposed himself and masturbated in her presence and his sister's. He had a voracious appetite, bit his nails, and wet his bed. He made good contact with the examiner, but he was unable to stop handling objects on the examiner's desk for more than a minute. The father, the dominant member of the household, had some understanding for the boy but beat him violently. The mother suffered from anxiety and conversion hysterical symptoms, and hated the boy.

He took thyroid (3 gr. daily) for one year. All endocrine symptoms cleared up

without dieting. In spite of prolonged psychotherapy he stole cars, was arrested and paroled 3 times. He finally took a job and became the only wage-earner in the family, of which both he and his family were proud. He was followed for 5 years.

The chief determinant of this patient's psychopathologic disturbance was of a hyperkinetic nature, but the picture was colored by environmental influences (rejection by the mother, over-severe father, oedipal wishes, sibling rivalry).

Case 6. A.B. became obese at the age of 12 years. The smallness of this patient's genitals was first noticed when he was 11 years. When first seen at the age of 13 years his height was 66 in.; the span of his arms was 70 in.; lower measurement 34 in.; his shoulders and hips were equally wide (13½ in.). He weighed 177 lb. (27 per cent above the median); his genitals were underdeveloped. His B.M.R. was—15.

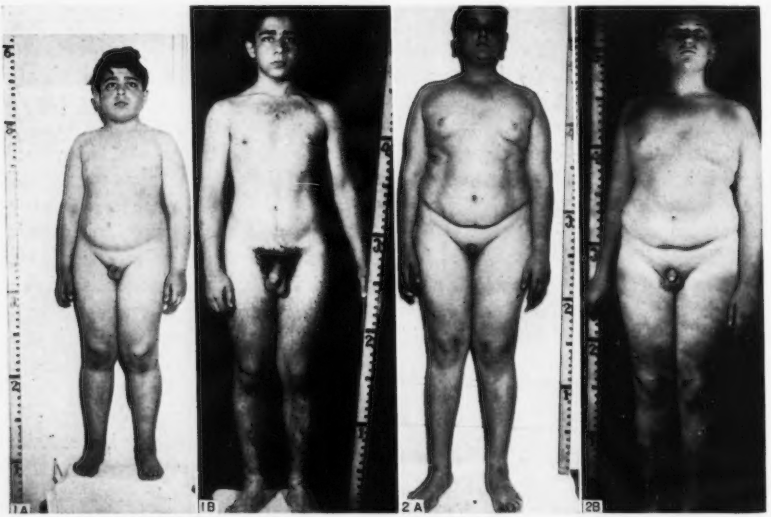

Fig. 1. *A. Case 1,* aged 10½ years. *B,* aged 15 years. Spontaneous correction (with thyroid).
Fig. 2. *A, Case 7,* aged 14 years. *B,* 9 months later, with APL and thyroid.

The neurologic examination disclosed unequal associated arm movements between the left and right side, and reaction of convergence.

He had been moderately overactive all his life. In the examiner's office he was always engaged in some activity. He was rather emotional and talked much, his speech approximating at times a flight of ideas. He had no friends. He was the youngest of 3 siblings. The family's attitude towards him was good, although they considered him a 'little crazy.' He presented no serious problem in supervision.

The endocrine symptoms cleared up under the administration of follutein and thyroid (3 gr. daily) and dieting. He received no psychotherapy. There was no change in his behavior in 2 years.

A Case with Circumscribed Depressive-Hostile Reaction

Case 7. A.M. became obese (fig. 2) at the age of 9 years. When first seen at the age of 14 years his height was 64 in.; the span of his arms 66½ in.; his lower measurement 34½ in. He displayed genu valgum and obesity accentuated over the upper and lower girdle. He weighed 165½ lb. (31 per cent above the median).

His penis was very small with a deep groove at its root; his testes were bilaterally undescended; the scrotal folds almost resembled labia majora; the hair-line of his scalp was low. His B.M.R. was —29. For the past 2 years, following an attack of scarlet fever, he has had an insufficiency of the mitral valve, with enlargement of the heart but without decompensation. The neurologic examination disclosed generalized muscular hypotonia.

Both testes descended on the 10th day of injections of follutein. Thyroid (2 gr. daily) was stopped after 2 months. The obesity persisted.

A psychopathologic survey, made after correction of the endocrine symptoms, revealed the following results. In spite of the marked endocrine dyscrasia, he had been very active in sports, and no behavior or mood disturbance appeared up to the time of obesity at the age of 9. Then he could not compete very well and was teased by his playmates. He said "I had weeping spells. I thought of killing myself." Both parents and the patient escaped conscious knowledge of the extreme genital underdevelopment until he was 11; then other boys remarked on his condition in the shower-room. He said: "Following this I would get angry at other boys for no good reason and would throw things at them." Neither the depression nor the hostile attitude was severe enough to disturb the environment. The latter reaction disappeared spontaneously after the correction of the genital disturbance. He was followed for 1½ years.

The patient's psychopathology represented sharply circumscribed reactions first to late obesity (at 9 years of age) and its concomitant impairment of competitive efficiency, and then to the inescapable knowledge of the under-development of his genitals.

Cases with Mixed Reactions to Injurious Environment and to Somatic Deficiencies

Case 8. J.G. became obese at 4 years of age and was stoutest at the age of 8½ years (115 lb.). When first seen at the age of 9½ years his height was 59 in.; the span of his arms 59 in.; lower measurement 32 in. He displayed genu valgum, excess fat deposits about the upper and lower girdle, somewhat small testes and a circular groove at the root of his penis. He weighed 108 lb. (11 per cent above the median). His B.M.R. was —13. The neurologic examination disclosed absence of reaction of abduction and lack of maintained muscle tone; the previously elevated arm sank below the horizontal.

He has always been 'clumsy, slow and weak' and even now frequently stumbled and fell, and dropped things. He chewed sand and pieces of wall as a young child. He had a fear of dogs, of the dark and of 'people in the closet' from the age of 4. He wanted to play with other children, but fought with them because they called him fat and sissy, and played pranks on him. He would attack his younger brothers violently, and once tried to choke one of them. He never urinated in the presence of other boys, and did not know that his genitals were small. He masturbated, had fits of anger, and of depression, wanted to be with his mother, but was stubborn and disobedient. The father never ate with the family, was cruel to the patient, called him clumsy and stupid. He had beaten him up as a child when he fell. The mother was unhappy because of her husband; at times she would feel 'desperate' over the patient's 'foolish talk' and over his fights with his brothers, but she felt sorry for him. The test on the patient showed 93 ideational, 82 motor intelligence.

He took thyroid substance (2 gr. daily) without appreciable change in the endocrine and neurological status in 2 years. Psychotherapy was partly successful.

The father was entirely uncoöperative and the patient's motility disturbance could not be changed; but the mother handled him better, and the boy himself found his lot more bearable.

The patient's psychopathologic manifestations (depression, violence, over-dependence and severe anxiety) represented reactions to the environmental situation, to circumscribed deficiencies of function and to pathophysiologic disturbances.

Case 9. J.S. was born obese. When first seen at the age of 4½ years his height was 41 in., with normal skeletal proportions; he weighed 56 lb. (32 per cent above the median), his testes were small and movable, his penis small with redundancy of the skin. He executed a movement with his head, about one or two every minute, characteristic of spasmodic torticollis (present since infancy). His mother was over-anxious and much concerned about his tic, and the boys made remarks about it. He has been irritable and assaultive for 2 years. This represented a reaction of the total personality to a circumscribed deficiency, accentuated by the maternal attitude. He was seen by the author twice.

Case 10. R.F. had been obese since infancy. When first seen at the age of 13½ years his skeletal proportions were eunuchoid. His left testis was undescended; his penis small with redundancy of the skin. His B.M.R. was —7. The neurologic examination disclosed convergence reaction, lack of neck-arm response to the turning of the head and choreo-athetoid movements of the outstretched arms.

He had been active in sports, but a problem in management all his life. He beat up boys who called him fat, and was a disciplinary problem in school and stole small objects. Recently he had nightmares, screamed, and walked in his sleep. He was not over-active at the examination. The quotient of his ideational intelligence was 86, his motor intelligence 108. His mother, the dominant member of the household, was domineering; she always scolded, often humiliated and never praised him. She preferred the older brother (3 years patient's senior).

There was prompt genital response to administration of follutein. His obesity persisted. No dieting was attempted, to avoid untruthfulness. At the age of 15½ years his height was 66 in.; the span of his arms 67½ in.; his lower measurements 35 in.; his weight 150 lb. (27 per cent above the median). There was good psychotherapeutic response. He was followed for 2½ years. The patient's psychopathology represented a reaction to the maternal relationship and to his bodily deficiencies.

Case 11. J.K. was referred to the author by Dr. Isabel S. Beaumont, who had had the patient under psychoanalytic treatment for 2 years for a severe compulsion neurosis and a high-pitched vocal tic. He would hop on his left leg, turn his head to the right and raise his right shoulder. There was diurnal and nocturnal enuresis. He prayed compulsively: "Dear God, do not let any harm befall mother." He beat up defenseless children without provocation. He was peculiarly without skill in any constructive work with his hands. He masturbated and rocked himself. All of these symptoms with the exception of the last two, cleared up under psychoanalysis. The patient, however, was still concerned about his small penis and his testes which "did not stay down." The physical symptoms were first noticed when he was 5 years old. His left testis descended at the age of 9 years.

When first seen at the age of 12 years his height was 58¾ in., the span of his arms 59 in.; lower measurement 31½ in. He displayed genu valgum. He had a small penis with redundancy of the skin; mobile testes; low hair-line of the scalp, and a freckled face. He weighed 110 lb. (19 per cent above the median). His B.M.R. was —14. The neurologic examination disclosed stronger associated movements in the left arm than in the right; slight reaction of convergence; lack of main-

tained muscle tone in the right arm; absence of neck-arm response on the left side; horizontal nystagmus in extreme lateral positions of the eyes.

After the first injection of follutein, the patient's anxiety and some of his compulsive symptoms returned. The injections were discontinued. There was no change in the patient's somatic condition a year later. He maintained his previous psychological improvement. This patient's compulsion neurosis completely overshadowed the psychopathology connected with his somatic condition.

Case 12. L.C. had been obese since infancy; his genitals were small up to the age of 13 years, then they developed spontaneously. When first seen at the age of 19 years his height was 66 in.; his weight was 220 lb. (37 per cent above the median); his genitals were of normal size. He showed an excess of hair all over the body. His B.M.R. was +2.

He has chewed pencils, the lapel of his coat, pieces of wood, etc., as far back as he could remember. When the father called him fat he "ate more out of spite." His mother had an exaggerated sense of cleanliness and nagged him. She was the stricter of the parents, but the patient liked her better. He had a sister 2 years his senior. Two years ago when the patient's mother went to the country for the first time in the patient's existence, he said: "I stopped eating by accident while mother was away." During this dieting he developed 'shaking on the inside,' 'ticking and shaking on the back,' and anxiety attacks: "I felt as if the earth is falling on me, then I begin to run." He had nightmares. The symptoms continued even after he stopped dieting and after his mother's return. There was some improvement in his symptoms in a month and a half of treatment. He then changed to another hospital.

The patient was unable to solve the emotional problem of his endocrine dyscrasia because of the poor environment; the neurosis finally resulting overshadowed his special reaction to his deficiencies.

Cases with No Reaction or Mild Reaction to Their Deficiencies, with Relatively Good Environments

Case 13. M.W. when first seen at the age of 12 years was 59¼ in. high; span of his arms 60½ in.; lower measurements 32 in. He displayed genu valgum; girdle adiposity; small testes, and small penis with a circular groove at its root. He weighed 120 lb. (25 per cent above the median). His B.M.R. was —24. He suffered from chronic rheumatoid arthritis for the past 6 months. In spite of the definite signs of adiposogenital dystrophy, an over-anxious although not over-protective mother, and a less strict father, the patient was free from psychopathological manifestations, and up to the onset of arthritis had been captain of the football team of his class. He stated on close questioning that he "felt bad and felt like crying" when he first found out about the smallness of his genitals; but this reaction did not lead to any further disturbances.

The glandular symptom as well as the arthritis cleared up under endocrine treatment (thyroid up to 4 gr. daily, and P.U. extract). He was followed for 1 year.

Case 14. J.N. had been obese since infancy. When first seen at the age of 4 years he presented a small penis with redundancy of the skin, bilaterally undescended testes and muscular hypotonia. There were no neurotic manifestations.

The testes descended promptly after the administration of P.U. extract.

Case 15. S.G. had been obese since infancy. He was operated on for bilaterally undescended testes at the age of 4½ years without success. When first seen at the age of 8 years his height was 47 in. with normal skeletal proportions. He weighed 71 lb. (28 per cent above the median). He showed operative scars in the inguinal

region bilaterally. His penis was of normal size, but the scrotal sack empty. He was an active boy, of the 'tough' kind, good in sports. He presented no psychopathology. He received maximal doses of follutein for 2 months, but the testes, evidently because of adhesions, did not descend. There was no change in his condition after 1 year.

Case 16. S.M. had convulsions at the age of 9 years for obscure reasons; following this he became obese. When first seen at the age of 15 years his height was 64 in.; span of his arms 64 in.; lower measurements 34 in. He showed girdle adiposity and weighed 126 lb. (8 per cent above the median). His genitals were of normal size. The hair growth was adequate in the pubic and axillary region. There were no abnormal neurologic findings.

He has always been active in sports and never shy or over-dependent. He was on good terms with his father and brother, 2 years his junior. His mother was overanxious but never restricted his activities. He bit his nails, had fear of the dark up to the age of 9 years. He had 'felt bad' about the smallness of his genitals but "he knew his parents did everything about it."

There was a gradual increase in the amount of hair growth under follutein and thyroid medication in the 3 years during which he was followed. No psychotherapy was given.

A reaction of the total personality to the circumscribed deficiencies was present but mild. Minor symptoms of a phobic nature were present. The environment was relatively good.

DISCUSSION

The problem of the pathology of adiposogenital dystrophy in general is still unsolved, as regards both the structures primarily damaged and the essential nature of the dysfunction. (For reports on human beings see 2, 3, 9, 15, 16, 17, 18, 22, 23, 24, 25, 26, 27, 28, 29, 30, 32. For animal experiments see 18, 19, 20, 21.)

There are no autopsy reports in the literature on the type of juvenile adiposogental dystrophy which forms the subject of this study, not excepting Hueters' (33) and Morgenstern's (34) case reports. Therapeutic effectiveness of P.U. extract makes it probable that at least the anterior lobe of the hypophysis is involved. In postencephalitic cases the syndrome does not undergo correction, as a rule. (For exception see Stern 31.) The concomitant neurological findings persisted after the correction of the endocrine signs for the length of time observed. They may be either the result of primary developmental pathology of structures adjacent to the pituitary gland or, at least some of them, secondary results of the glandular disturbance.

The effectiveness of P.U. extract in making the testes descend and initiating genital development is well established (35-42). Hypogonadism with descended testes but without obesity does not respond to the administration of P.U. extract. (See also Sexton, 37.)

As to psychopathology Weygandt (44) reports imbecility, restlessness and cheerfulness in his cases. They had very severe lesions of the central system. (See also Zondek 21, Kraus 18.) Molitch (45) states that the psychological difficulties of boys suffering from cryptorchism is caused largely by their reactions to their somatic difficulties. His cases responded to the administration of

P.U. extract. Rowe (46) reports on a male castrate (surgical) with profound mental depression and a lack of ambitions. Later the patient married and proved to be potent; he took up his profession again with success. This case illustrates that the psychological reaction to a bodily defect may completely overshadow the direct effects of pathophysiologic condition. Sylvester, Ginsburn and Blackman (47) found that the behavior-pattern of their hyperactive children was altered with a change in environment (implying a psychogenic disturbance). Bond and Smith (48) report that even encephalitic hyperkinetic children can be slowly reëducated. (For brain pathology, found in postencephalitic hyperkinetic children, see 49, with further references.)

Levy found the majority of his cases with Fröhlich syndrome predominantly 'submissive' although most of them showed 'aggressive' features, also. The percentage of 'submissive' behavior in the adiposogenital dystrophy group was higher than in groups of children with obesity only, or with under-developed genitals only.[4]

In this study the existence of reactions of the total personality to circumscribed deficiencies was assumed on the basis of direct evidence: the patient's statements and reactions. The existence of excess 'oral drive' and of hyperkinetic disturbance was assumed on the basis of observation, of history (manifestations since infancy) and of relative refractoriness to psychotherapy, i.e., hyperactivity during interviews with the author in spite of repeated and good contact. The evidence for a physiological 'poverty of drive' is least direct. No automatic transformation of behavior was observed in this series with correction of the endocrine symptoms in markedly over-dependent patients. On the other hand, two of the cases in this paper (7 and *13*) were 'normally aggressive' boys in spite of a striking endocrine dyscrasia (but late appearance of obesity). Apparently, 'poverty of drive,' as it exists in adiposogenital dystrophy, must be combined with additional factors (obesity since infancy: *Case 2;* pampering home invironment: Levy's (50) cases) to make the patient overdependent.

SUMMARY

Non-neoplastic, non-encephalitic, juvenile adiposogenital dystrophy is a syndrome characterized by obesity, under-developed genitals, anomalies of the skeletal structure, and a tendency to low basal metabolism. The syndrome may correct itself spontaneously or can be corrected by the administration of P.U. extract (prolan). In either case, the genital development, once started continues and may proceed more rapidly than in the average normal boy. Neurologic disturbances may be limited to muscular hypotonia or may include clumsy motility, tendency to postural reflexes which deviate from the adult norm, intention tremor and inequality of the associated arm-movements, spasmodic torticollis, nystagmus. Psychopathological manifestations referable to the syndrome may be absent. When present, they are determined, *A).* By direct somatic influences: *a),* poverty of 'general drive'; *b),* marked 'oral drive,' (big appetite, tendency to chew); *c),* hyperkinesis (infrequent). *B).* By the reac-

[4] Levy correctly implies 'submissiveness' and 'aggressiveness' are clinical and social concepts.

tions of the total personality to localized somatic deficiencies (endocrine and neurologic) because of: *a)*, injured self-esteem (narcissistic injury); *b)*, inability to master functions adequately; *c)*, loss of parental love and sibling rivalry (environmental influences) because of the somatic deficiencies.

The chief psychopathological manifestations are depression, over-submissive, over-dependent and/or over-aggressive, hostile behavior disturbance. The psychopathology proper of the adiposogenital dystrophy is usually interwoven with psychopathology traceable to problems of development and of the family environment (transference neuroses) not directly connected with the syndrome. The best management of the condition consists of correction of the endocrine disturbance together with adequate psychotherapy at any age when the patient comes under observation. The psychotherapeutic results are satisfactory in the majority of the cases.

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CASE REPORTS

PITUITARY CACHEXIA?[1]

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CASE REPORT

V. K., No. I-2979, a white, divorced housewife, 29 years of age, entered Multnomah County Hospital on October 3, 1929, complaining of loss of weight, hunger, amenorrhea, a 'drugged feeling,' and weakness. She was perfectly well in 1921 when she was married. At this time she weighed 140 lb. (63.6 kg.) and had dieted and used various proprietary preparations in an unsuccessful effort to lose weight. Two months after she was married she became pregnant, but the husband was not thought to be the father, so she had an abortion performed. She has not menstruated since this abortion, and dates her loss of weight from this time. Libido disappeared, and she has not experienced an orgasm since. For a period at this time she was very thirsty and drank a great deal of water. An electrotherapist found sugar in the urine and told her she had diabetes. Until 1923 she followed the diet prescribed, but since that time she has eaten everything. Loss of weight has been progressive and extreme, notwithstanding a good appetite. In 1927 her teeth became loose and were extracted. Since 1927 she has had occasional attacks of weakness in the extremities lasting from one-half hour to one-half day, associated with numbness and tingling. She had measles, mumps, smallpox, and chickenpox as a child, and contracted gonorrhea in 1917.

Her father is alive and well, aged 60, as is her mother, aged 52. She has two sisters and one brother alive and well. No family history of insanity, tuberculosis, diabetes, or endocrine disturbances was elicited.

Physical examination revealed a woman 63 inches (160.0 cm.) tall, weighing 58 lb. (26.3. kg.). Her appearance was that of an extremely emaciated woman at least 75 years of age (fig. 1*A* and 1*B*). The blood pressure was 120 systolic and 75 diastolic, the pulse 74, the respiration 18, and the temperature 97.4°F. (36.3°C.). The hair was red, scanty, and dry. The face was thin and looked like parchment stretched over a skull. The pupils were equal, reacted to light and in accommodation, the visual fields normal. There was slight physiologic nystagmus to the left. The teeth were out, and the gums atrophic. There was a fistula leading from the left maxillary sinus to the gum margin. No abnormal pulsations, masses, or nodes were felt in the neck. The thyroid was not palpable. All of the muscles of the face and neck were atrophic, but could be used with a fair degree of strength. There were no fibrillary tremors. The chest was extremely emaciated, but respiratory movements were equal, and resonance not impaired. There were no rales or alterations in the breath sounds. The heart borders measured 3 cm. to the right of the sternum and 6 cm. to the left. No murmurs were heard. The aortic and pulmonic second sounds

[1] No review of the literature is given, since that is adequately covered by Lisser and Escamilla in a forthcoming paper.

were equal. The abdomen was markedly scaphoid and so emaciated that even the iliac fossae could be seen. There were no palpable masses. The liver, spleen and kidneys were not felt, notwithstanding the extremely thin abdominal wall. There was no tenderness to palpation. The back was so emaciated that a deep gutter was

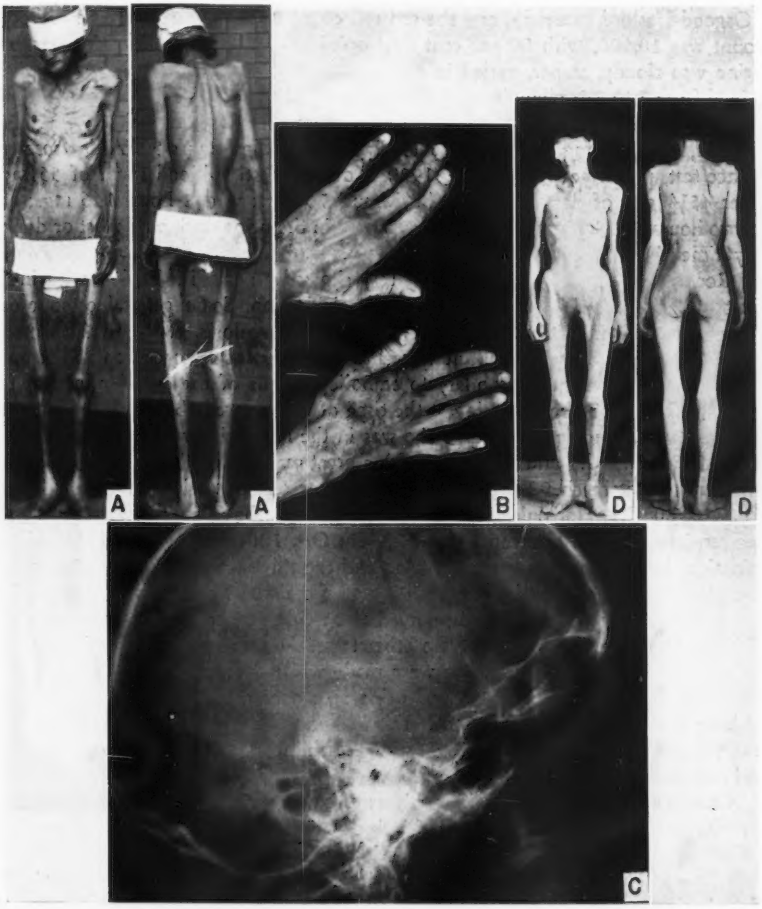

Fig. 1*A*, Front and back views of the patient, taken in Oct., 1929. Note the extreme emaciation and tissue atrophy without marked loss of strength, and the appearance of great age. *B*, Hands of the patient, photographed in May, 1933. Note that they resemble more the hands of a 75-year-old woman than those of a woman 33 years of age. *C*, Roentgenogram of the skull, taken on Oct. 10, 1929. Note the decalcification of the posterior clinoids and the normal size and shape of the sella. The plates, taken in May, 1933, were similar in appearance. *D*, Appearance of the patient in May, 1933, after 2½ years of a high calory diet.

visible between the ribs and the vertebral spines. The extremities were markedly atrophic, but her strength was surprisingly good, considering the small amount of muscle tissue present. The nails of both the hands and toes were somewhat recurved. The hands (fig. 1*B*) looked like those of an old woman. The skin was dry and atrophic. There was good pulsation in the radial, dorsalis pedis and posterior tibial arteries. The biceps, triceps, periosteoradial, patellar and Achilles reflexes were

present and equal. Axillary and pubic hair was present but scant. The uterus was atrophic. She seemed mentally alert and answered questions promptly and intelligently, but was extremely negativistic when coöperation in the physical examination was desired.

Laboratory Studies. The hemoglobin estimation was 12.7 gm. or 92.0 per cent (Osgood-Haskins method), and the red cell count was 4.55 million. The leukocyte count was 10,000, with 80 per cent neutrophils and 20 per cent lymphocytes. The urine was cloudy, amber, varied in specific gravity from 1.014 to 1.030, and contained from 1 to 2+ albumin with an occasional pus cell. The B.M.R. was —27 per cent. The fasting-blood sugar on Oct. 4, 1929, was 87 mg. per 100 cc. and, on Oct. 11, 1929, 76 mg. per 100 cc. The alkali reserve figure was 50. A dextrose tolerance test, performed on Oct. 30, 1929, showed a fasting-blood sugar of 55 mg.; after 45½ gm. of dextrose at one-half hour, 76 mg.; at one hour, 146 mg.; and at two hours, 143 mg. Dextrose did not appear in the urine during this test or during any time she was under observation.

Roentgenograms of the skull (fig. 1*C*) showed a normal sella, but with indistinct posterior clinoids probably due to decalcification. Some general osteoporosis was evident. Chest plates showed no evidence of tuberculosis.

A diagnosis was made of pituitary cachexia with extreme emaciation and atrophy of all organs and tissues secondary to embolic necrosis of the anterior lobe of the pituitary from an embolus arising at the time of the induced abortion.

Treatment. During the 55 days she was in the hospital her temperature was between 96° (35.5°C.) and 97° (36.1°C.) a great majority of the time. On only two occasions did it reach 98.6° (37.0°C.); whereas on 3 occasions it was as low as 95.0° (35.0°C.). Her pulse was between 60 and 75 the majority of the time, and her respirations were between 10 and 20. On Oct. 10th, during the B.M.R. determination, her respirations were 10/min., and the tidal air volume was 300 cc. She was placed on a diet of carbohydrate 200 gm., protein 100 gm., and fat 50 gm., but great difficulty was obtained in keeping her on this, as she constantly complained of being hungry and stole food from other trays. Attempts at analysis of accurately collected 24-hour samples of urine were made while she was on this diet, but it is extremely doubtful if the 300 cc. of urine sent over actually represents her total 24-hour volume. The total nitrogen of this 300 cc. volume was 2.27 gm.; urea nitrogen, 1.4 gm.; ammonia nitrogen, 0.33 gm.; creatinine, 0.24 gm.; creatine, 0.12 gm.; and uric acid, 0.31 gm.

Careful metabolic studies with analyses of stools, urine, and accurate control of her diet were planned, but because of lack of coöperation had to be given up. The patient would not save her specimens and constantly ate food from other persons' trays. It was only with the greatest difficulty that she was persuaded to permit the pictures shown in figure 1 to be taken, and then she insisted on having them taken in the pose and costume shown.

On Oct. 11th, she was placed on a 3,000 cal. diet, her weight having decreased to 55½ lb (25.2 kg.). On Nov. 20th, she weighed 62¼ lb. (28.3 kg.). On Nov. 18th, antuitrin-G was started in one-half ampule doses and changed, on Nov. 20th, to one ampule per day. On the morning of Nov. 20th, the patient reported a spontaneous orgasm during the night, stating that it was the first she had experienced since the onset of her illness; but she refused any further medication. On Nov. 27th, the patient signed her release and left the hospital against the advice of her physicians and family. Her appetite had been recorded as fair or good in the nurses' notes during her entire stay in the hospital.

The final diagnosis was pituitary cachexia with extreme emaciation and atrophy of all organs and tissues secondary to embolic necrosis of the anterior lobe of the pituitary from an embolus arising at the time of the induced abortion.

Efforts were made to see her at intervals at home, but she moved without leaving her address and was completely lost sight of by the author, until 1933, when it was discovered that she was in the tuberculosis ward of the Oregon State Hospital for the Insane with a diagnosis of psychopathic personality and tuberculosis, and had been there since Oct. 7, 1930. Through the coöperation of the superintendent and especially Dr. P. W. Bird, the additional photographs shown in figures 1*B* and *D* were taken. She had been placed in the tuberculosis ward for forced feeding because of her extreme emaciation, and not because of any clinical or roentgenographic evidence of tuberculosis at the time of her admission. Notwithstanding the forced feeding for 2½ years, her weight was still only 72 lb. (32.7 kg.). She was quite strong, however, and helped about the ward, although still extremely negativistic. She was classified as having a psychopathic personality, although no evidence of any of the classified psychoses was found.

In the roentgenograms taken May 31, 1933, there were evident, for the first time, soft shadows, suggesting recent conglomerate tubercles in the left apex. On several different occasions the note was made on her record at the State Hospital: "Appetite voracious. Attempts to steal food from other patients." Her weight was recorded monthly while in the State Hospital: in 1930 it ranged from 67 to 75 lb.; in 1931 from 61 to 71 lb.; in 1932 from 73 to 89 lb.; in 1933 from 72 to 80 lb.; in 1934 from 75 to 80 lb.; and in 1935 from 78 to 80 lb. She had one B.M.R. determination on Sept. 19, 1933 which was —31.6 per cent. No sugar or albumin was found in the urine in any examination while she was in the State Hospital. A dextrose tolerance test, performed on Dec. 14, 1932, showed a fasting-blood sugar of 64.5 mg. per 100 cc.; the blood sugar was 83.3 mg.; at one-half hour after 100 gm. of dextrose; at 1 hour, 81.6 mg.; and at 2 hours, 72.7 mg. Sugar did not appear in the urine during this test. She died on April 9, 1935.

Necropsy. Necropsy was performed on the same day, and revealed extreme emaciation, wasting of all body tissues, dry skin with parchment-like consistency, absent teeth, atrophic mammary glands, and an ischiorectal abscess with rupture both externally and into the rectum. The usual mid-line incision revealed an extremely small amount of yellow subcutaneous fat. The peritoneal cavity contained a large amount of green, purulent fluid. The small intestine was distended and hyperemic, and the peritoneum was covered with fibrin. A small perforation was found in the ileum and a fistula opening from the rectum into the abscess cavity. The liver, spleen, lymph nodes, and kidneys were described as normal. The uterus was very small and atrophic. The fallopian tubes were thickened, and the ovaries were bound to them by dense adhesions. The ovaries were very small, measuring 1.5 cm. x 0.5 cm. The heart measured 7.5 cm. in its greatest diameter. The left lung was densely adherent. The right lung was free in the pleural cavity. There were several caseous nodules in the upper and middle lobes of the right lung. The left lung showed numerous miliary tubercles. The pituitary gland appeared grossly normal and weighed 760 mg. Microscopic examination of the pituitary, adrenals, and thyroid showed no significant deviations from normal. The ovaries were atrophic, and the lung section showed miliary and conglomerate tubercles with very little evidence of old tuberculosis. The pathologic diagnosis was acute, purulent peritonitis, perforation of the ileum, bilateral conglomerate and miliary tuberculosis of the lungs, and ischiorectal abscess.

COMMENT

Clinically, this patient showed every symptom and sign that has been described for pituitary cachexia, including amenorrhea, loss of libido, extreme emaciation with atrophy of the organs and tissues, loss of teeth, decrease in axillary and pubic hair, psychopathic personality, low basal metabolism, low dextrose tolerance curve without glycosuria, and failure to gain weight on a high caloric diet. Notwithstanding this, an essentially normal pituitary gland was found at necropsy.

Anorexia nervosa has been thought by some to be capable of producing all the symptoms and signs of pituitary cachexia, but this patient was under direct observation over a period of more than 4 years, during all of which time she was noted to have a voracious appetite without diarrhoea, vomiting, fever, or elevated metabolism to account for her loss of weight. It is true that she had tuberculosis at the time she died, but the onset of the tuberculosis was not until the patient had been in a tuberculosis ward for 2 years, nor until 4 years after she was first under observation, nor until 12 years after the onset of the symptoms, and the tuberculous process was relatively acute and not the immediate cause of death.

SUMMARY

A patient having a complete clinical and laboratory picture of pituitary cachexia with a normal pituitary gland is described. No explanation for this syndrome was discovered, either during life or at necropsy.

THE INDUCTION OF PREMATURE PUBERTY WITH ANDROGENIC SUBSTANCE

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In recent years numerous reports concerning the treatment of cryptorchidism and hypogenitalism with the use of various gonadotropic hormones have appeared in the literature. Most reports have dealt with the use of gonadotropic hormones either from pregnancy urine, placenta, or beef pituitary and have been used chiefly for the treatment of the afore-mentioned conditions in young males.

Recently, Thompson (1) and his associates have reported on the production of precocious puberty and hypertrophy of the genitalia in males receiving gonadotropic hormone for the treatment of cryptorchidism. In their cases, however, these conditions were produced only after large doses of hormone had been administered over long periods. In our own experience (2) covering a period of over 6 years, during which time we have treated a considerable number of males with hypogenitalism, some with and some without cryptorchidism, we have not observed the development of premature or precocious puberty or abnormal hypertrophy of the genitalia following the dosage administered, namely: APL hormone 100 to 200 R.U. 3 times weekly, total dose not in excess of 7000 to 8000 R.U. in any one course of treatment. Anterior pituitary gonadotropic hormone was administered to many of our patients in doses from 1 to 2 cc. 3 times weekly for several months without untoward results. In some of our older boys, however, who were at the age when puberty usually begins to appear, the administration of the hormones apparently accelerated the development of pubertal changes.

Recently, considerable attention has been given to the chemistry, physiology and therapeutic application of the testis hormone. Many claims have been made as to the clinical value of this hormone. McCullagh and his associates (3) and Hamilton (4) have shown improvement in adult hypogonadism, and Hamilton (5) has produced penile erections experimentally in the monkey and in impotent males with androgenic substance. Various manufacturers have made claims for their products relative to the improvement of sexual impotence, infantilism, hypogonadism, cryptorchidism, etc., and have mentioned little in so far as the limitations and contraindications for administration of the hormone are concerned. In administering male hormone to young males we should be cognizant of several facts. It is true that puberty may be accelerated, as characterized by the rapid development of the secondary sex characteristics; nevertheless, it has been shown experimentally that at least one fraction of the

testis has a depressent action upon the hypophysis (6), and this fraction may be present in the testis hormone. Therefore, it is possible that secondary atrophy of the gonads may take place as a result of decreased secretion of pituitary gonadotropic hormone.

Lower (6) states that "there is an antagonism between the sex hormone and the hypophyseal gonad-stimulating hormone, and that an excess of either the male or the female gonadal hormones depresses the production of the hypophyseal hormone." On this basis, massive doses of estrogenic substance have been used in the treatment of hyperpituitarism (acromegaly). More recently, Hamilton and Wolf (7) have shown experimentally that synthetic androgen (testosterone propionate) lessened the gonadotropic properties of the rat's pituitary.

In so far as the treatment of infantilism, secondary hypogenitalism and cryptorchidism are concerned, androgenic substance should have no therapeutic value. Moore (8) states that the testis-hormone function is related primarily to reproduction by virtue of its action on the accessory reproductive organs and the development of the secondary sex characteristics to carry on sex function. He states that there is little evidence that the testis hormone in man exerts any beneficial effect aside from its control over the accessory reproductive organs. Hormones produced by the gonads are not gonadal stimulants, and in large doses may prove to be injurious to the gonads.

It is felt that the report of 2 instances of abnormal acceleration of puberty and genital hypertrophy in two boys with sexual underdevelopment should be reported as a warning against the promiscuous use of potent androgenic substance in childhood.

There is considerable difference of opinion as to the definition of puberty—at what age it begins and when it ends. Heritage, climate, race and environment are all factors. It probably begins in males between 14 and 15 years of age and ends between 17 and 18 years of age (adolescence) and is characterized by the development of secondary characteristics and the ability to reproduce. Even though puberty is occasionally reached as early as 12 or 13 years of age, our patients showed no evidence at the time of treatment that would indicate a spontaneous premature puberty.

CASE REPORTS

Case 1. I.S., aged 11½ years. At the age of 10½ years this patient's height was 50⅝ in., and his weight was 62 lb. He was undersized and his genitalia were small (fig. 1). Physical findings were normal. Roentgenograms revealed normal sella and normal epiphyseal development for age. Blood chemical constituents and B.M.R. were within normal limits. Between 10½ and 11½ years of age he received several courses of injections of anterior pituitary growth hormone[1] and desiccated anterior pituitary and thyroid extract orally. At 11½ years, his height was 53¾ in. and his weight 71½ lb., a growth of 3⅛ in. and a gain of 9½ lb. There was no remarkable increase in size of the external genitalia (fig. 1*B*). The prostate and seminal vesicles were not enlarged. Beginning at 11½ years he received intramuscular injections of androgenic substance twice weekly.[2] He received a total of 21 injec-

[1] Phyone, Wilson Laboratories.
[2] Oreton (testosterone propionate), Schering Corporation, supplied by Dr. Max Gilbert.

tions of 5 mg. each. At the time he received his 10th injection his voice was deeper, pubic hair was present, the genitalia were larger and he had been having frequent erections. He was given a rest period of 6 weeks and then 11 more injections were administered. At the age of 12¼ years (fig. 1*C*), approximately 9 months from the time of his first injection and 4½ months after his last injection, pubic hair was abundant and the genitalia had increased remarkably in size. The penis was greatly hypertrophied. Erections were frequent, and seminal emissions occurred one to two times weekly. The prostate and seminal vesicles had increased in size. Sparce hair was present in the axilla, on the face and upper lip. His height was 56½ in. and his weight 86¾ lb., a growth of 2¾ in. and gain of 15¼ lb. in 9 months. His muscular development was improved. Roentgenograms of the extremities did not reveal premature closure of the epiphyses.

Case 2. E.P., aged 10⅔ years. At the age of 10 years this patient received a total of 2700 R.U. of APL for undescended testes (fig. 1*D*). The testes descended, but during the following 8 months the genitalia remained underdeveloped. At 10 years 8 months he was overweight, with excessive deposit of fat over abdomen and hips. His height was 59½ inches and his weight 129 lb. Genitalia were underdeveloped. Prostate was small and soft, and seminal vesicles were infantile. His voice was soft, his habitus was feminine and there was no pubic or axillary hair (fig. 1*E*). He was given androgenic substance[2] 5 mg. twice weekly for a total of 28 injections, with a rest period of 2½ months following the 18th injection. At the end of his 18th injection, his penis, scrotum, and testes had grown considerably, pubic hair was present and he was having frequent erections. At the time the second course of 10 injections was finished, at 11 years 2 months, approximately 6 months after the first injection was given, his genitalia had grown larger, pubic hair was abundant and axillary hair was present. Erections were frequent. The prostate and seminal vesicles had grown considerably. He had had seminal emissions on two occasions, his voice was deeper and he was showing interest in girls. There was no change in his fat distribution. His height was 61 in., weight 133 lb. (fig. 1*F*). Roentgenograms of the extremities revealed normal epiphyseal development.

COMMENTS

The ability of androgenic substance to produce premature puberty labels it a dangerous drug, in so far as its administration to children is concerned. The undesirability of premature sexual maturation is obvious and does not need further comment. It is true that not all individuals will respond similarly to a hormone; and the amount of hormone that causes excessive stimulation in one may have little or no effect upon another. However, since there is no method by which to prophesy an individual's response to a hormone, the hormone must be administered cautiously.

The amount of hormone administered to both of the boys might be considered small. *Case 1* received only 21 injections over a period of approximately 16½ weeks, with a 6-weeks' rest period after the 10th injection, and *Case 2*, 28 injections of 5 mg. each over a period of 6 months, with a 2½ months' rest period after the 18th injection. Nevertheless, marked stimulation occurred with a rapid development of secondary sex characteristics in both boys. Furthermore, in the case of *I.S.* (fig. 1*C*), the genitalia were greatly hypertrophied. There may be some question raised as to the part played by the

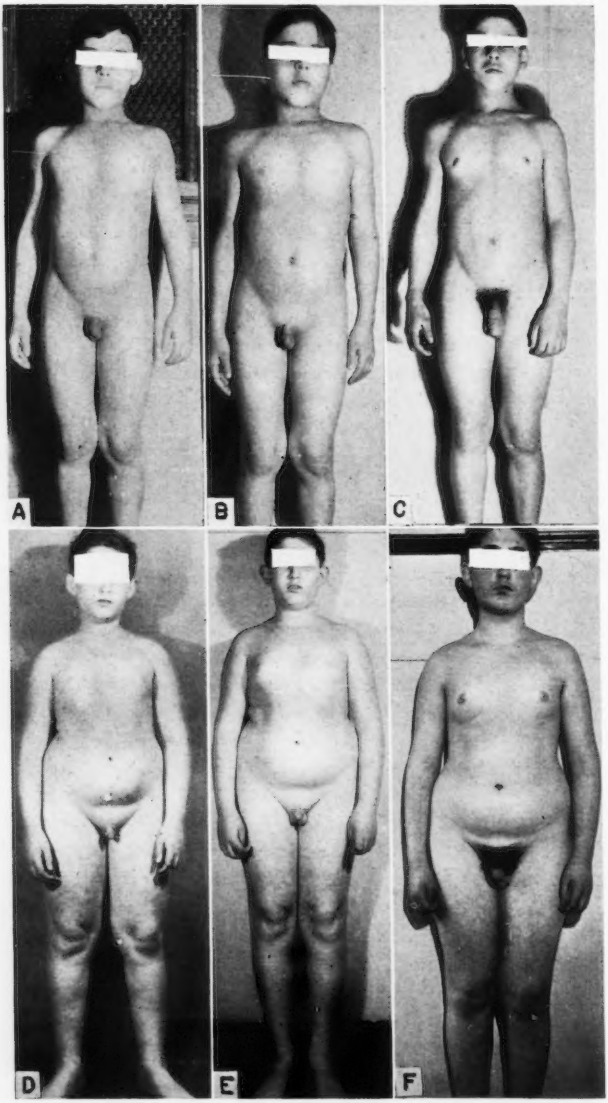

Fig. 1*A*, *Case* 1, aged 10½ years. Ht. 50⅝ in., wt. 62 lb. Undersized with small genitalia. 1*B*, aged 11½ years. Ht. 53¾ in., wt. 71½ lb. Growth of 3⅛ in. and gain of 9½ lb. in 1 year following several courses of phyone, desiccated anterior lobe pituitary and thyroid extract. No obvious increase in size of genitalia. 1*C*, aged 12¼ years. Ht. 56½ in., wt. 86¾ lb. A growth of 2¾ in. and gain of 15¼ lb. in 9 months following androgenic therapy. Note the remarkable increase in size of genitalia (hypertrophy) the increased muscular development and the more mature facial appearance.

Fig. 1*D*, *Case 2*, aged 10 years. Obesity and bilateral cryptorchidism, 1*E*, aged 10⅔ years. Ht. 59½ in. wt. 129 lb. Obesity; testes descended; genitalia small. 1*F*, aged 11 years 2 months. Ht. 61 in., wt. 133 lb. A growth of 1½ inches and gain of 4 lb. in 6 months following androgenic therapy. Note remarkable increase in size of genitalia, abundant public hair and mature facial expression following androgenic therapy. No change in distribution of fat.

pituitary growth extract and the desiccated anterior pituitary lobe administered primarily to the first patient and to the part played by the pregnancy urine extract administered to the second patient. However, it is not likely that the amount of gonadotropic principle which might have been present in the first 2 preparations, or the relatively small dose of antuitrin-S administered to the second patient (2700 R.U.) in the light of our previous experience, could have been important factors in the production of premature puberty. At the time these boys were treated, 5 mg. was the largest individual dose of androgenic substance commercially available, and the dose administered to these patients was merely a matter of personal selection. As there is no clinically established dose of any androgen for the treatment of hypogenitalism in children, and with the marketing of androgenic substance in larger individual doses, a greater degree of conservatism must be shown. We must also keep in mind Moore's (8) warning that the administration of testis hormone in sufficient concentration may ultimately be injurious to the gonads; probably by a suppressive action upon the hypophysis. For this reason, these two patients will be observed repeatedly for any new changes.

SUMMARY

Premature puberty was induced in two sexually retarded boys, aged 11½ years and 10⅔ years respectively, by the intramuscular administration of androgenic substance (testosterone propionate). In addition to the establishment of premature puberty in the case of the 11½-year-old boy, abnormal genital hypertrophy resulted. At the onset of treatment both boys presented small genitalia which were not considered dystrophic. In view of the results obtained in these two patients, the danger of administering potent androgenic substance to young individuals should be realized.

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ASSOCIATION NOTICE

At the twenty-second annual meeting of the Association in San Francisco, June 13 and 14, 1938, the following revision of the By-Laws[1] of the Society was recommended by the Council and was adopted by the members at the annual business session.

THE ASSOCIATION FOR THE STUDY OF INTERNAL SECRETIONS BY-LAWS

ARTICLE I.—*Name and Object*

The name and objects of this Association shall be as set forth in the Articles of Incorporation. The organization shall be conducted for scientific purposes, for the advancement and promulgation of knowledge regarding the internal secretions and for the facilitation of personal relationship among investigators in the subject of endocrinology.

ARTICLE II.—*Membership*

SECTION 1. The Association shall consist of active members only. Of these there shall be two classes, annual members and life members.

SECTION 2. *Eligibility:* Any qualified physician or biologist in good professional standing shall be eligible for nomination to annual membership. Annual members of high professional standing may, upon invitation by the Council, become life members upon payment of one hundred dollars, which sum shall be in lieu of annual membership assessments.

SECTION 3. *Nomination and Election:* Nominations for membership shall be made and seconded by members of the Association on blanks furnished by the Secretary, and shall be submitted to the Council. The Council shall determine eligibility and shall vote by ballot upon nominations. A majority of votes cast shall constitute election.

SECTION 4. *Resignation, Forfeiture and Expulsion:* The resignation of any member in good standing may be accepted at any annual meeting of the Council, providing his dues have been paid in full for the current year.

Any member whose annual assessment is in arrears one month after the sending of a second notice to his last known address by the Secretary Treasurer shall automatically forfeit his membership. Delinquent members may be reinstated by the Council providing all indebtedness to the Association is discharged.

Upon the recommendation of the Council any member may be expelled by a majority vote of the members present at an annual meeting of the Association, provided that a copy of the charges against the member in question and a notice of hearing thereon by the Council shall have been given him at least thirty days before the annual meeting.

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[1] The Constitution of the Association comprises the Articles of Incorporation, as set forth in the certificate recorded with the Secretary of State of the State of Delaware on January 31, 1918.

These Articles of Incorporation grant broad powers to the Association; give it perpetual existence; exempt entirely the private property of members for the payment of corporate debts; specify the general qualifications for membership; and authorize the Society to make and alter By-Laws for the regulation of its activities.

ARTICLE III—*Meetings and Quorum*

SECTION 1. *Annual:* The annual meeting of the Association shall be held at such time and place as shall be determined by the Council.

SECTION 2. *Special:* Regional branches of the Association may be authorized by the Council, upon petition by ten members. These may hold special scientific meetings as desired and elect their own officers.

SECTION 3. *Quorum:* Fifteen members shall constitute a quorum for the transaction of business at any annual meeting.

SECTION 4. *Council Meetings:* At least one meeting of the Council shall be held at each annual meeting of the Association. In the interval between annual meetings the President may on his own volition or at the request of two members submit questions by mail to the members of the Council for their consideration and decision.

ARTICLE IV—*Officials*

SECTION 1. *Officers:* The officers shall be a President, a President-Elect, a Vice-President and a Secretary-Treasurer, who shall be elected annually for a term of one year by the members of the Association. The officers shall enter upon their duties at the close of the annual meeting at which they are elected. The powers and duties of the officers shall be such as usually devolve upon their respective positions.

SECTION 2. *Council:* The officers so elected and nine additional members, three of whom shall be elected at each annual meeting, shall constitute the Board of Directors of the Corporation and shall be known as the Council. The term of office of members of the Council shall be three years. The President of the Association shall be ex-officio the Chairman of the Council. The Secretary-Treasurer shall be ex-officio the Secretary of the Council.

SECTION 3. *Powers:* The general management of the Association shall be vested in the Council. It shall regularly perform the ordinary duties of a board of directors of a corporation and shall possess all the powers conferred upon the directors by the Charter of Incorporation. The Council shall have power at any time to appoint any committee it may deem necessary and to delegate whatever powers necessary to such committees.

ARTICLE V—*Nomination of Officers*

SECTION 1. *Nominating Committee:* The Nominating Committee shall consist of three members appointed for the ensuing year by the newly-elected President and accepted by the Council. The President shall designate one member to be Chairman of the Nominating Committee.

SECTION 2. *Nomination of Officials: A).* The Nominating Committee shall make at least one nomination for each of the four offices and for each of the positions on the Council and on the Publication Board to be filled by vote of the members. Any member of the Association may submit nominations to the Nominating Committee for its consideration. *B).* The nominations of the Nominating Committee shall be transmitted to the Secretary at least sixty days before the annual meeting at which they are to be considered. *C).* The Secretary shall send to each member, at least four weeks before the annual meeting, a printed ballot containing the list of nominees and space for such additional names as the member wishes to propose, and at the same time shall notify the members that they may vote by mail by returning the marked ballot to the Secretary before the beginning of the business session at which the elections are to take place.

SECTION 3. *Elections:* At the annual meeting the Secretary shall present to the Tellers, appointed by the President, the ballots submitted by the members. The Tellers shall count the votes and announce the results at the business session. A majority of votes cast shall be necessary to elect an official. In case no candidate shall have received a majority of votes the office in question shall be filled by majority vote of those members present at the business session.

SECTION 4. *Filling of Vacancies:* The Council shall fill all vacancies in elective positions except such as occur at an annual meeting of the Association.

ARTICLE VI—*Financial*

SECTION 1. *Dues:* The dues shall be the annual cost of subscription to ENDOCRINOLOGY for members, plus an annual membership assessment which shall be determined by majority vote at the annual meeting, upon recommendation by the Council.

SECTION 2. *Expenditures:* No expenditures from the general funds of the Association except those required in the performance of the ordinary duties of officers shall be made except by vote of the Society or the Council.

ARTICLE VII—*Publications*

SECTION 1. The official organs of publication of the Association shall be the bulletin ENDOCRINOLOGY and such other periodicals or books as from time to time the Association may authorize. Ownership of these shall be vested in the Association.

SECTION 2. The editorial and financial management of all publications of the Association shall be vested in a Publication Board consisting of an Editor and six other members. The members of the Publication Board except the Editor shall be elected by the members of the Association at the annual meeting. Two members of the Publication Board shall be elected each year and the term of office of each shall be three yearse. The Editor shall be appointed by the Council and shall act as Chairman. The Publication Board shall be empowered to enter into contracts and agreements with individuals or organizations, as agents of the Association, for the manufacture and business management of the publications. The Publication Board shall submit to the Council each year a report of its activities and of the financial condition of the publications.

ARTICLE VIII—*Papers at Scientific Sessions*

The President and Secretary shall be authorized to arrange programs for the scientific sessions at the annual meeting. The manner of selection of papers to be presented and the time allowed for presentation shall be determined by the Council.

ARTICLE IX—*Changes in By-Laws*

SECTION 1. These By-Laws, after having been approved by the Council and adopted by the Association, may be amended at any annual meeting as hereinafter provided.

Proposed amendments to the By-Laws must be sent to the Secretary at least four weeks before the date of the annual meeting at which they are to be considered. The Secretary shall promptly transmit them to the members of the Council. At the annual meeting the Council shall consider the proposed amendments and make recommendations to the members at the business meeting of the Association. A majority of the votes of members at the business session of the annual meeting shall be necessary for adoption of an amendment.

CURRENT ENDOCRINE LITERATURE

COLLABORATORS

Fuller Albright
Wayne J. Atwell
Geoffrey Bourne
Israel Bram
John C. Burch
H. J. Deuel, Jr.
John C. Donaldson
Maurice H. Friedman
Murray B. Gordon
Arthur Grollman
Frank A. Hartman
E. C. Hamblen
F. C. Koch
Edward Larson
J. T. Lewis
Joseph M. Looney
A. E. Meyer
Warren O. Nelson
J. P. Pratt
A. T. Rasmussen
Elmer L. Sevringhaus
L. B. Shpiner
D. L. Thomson
R. L. Zwemer

ADVANCE ABSTRACTS

The influence of estrogens in egg yolk upon avian blood calcium. Altmann, M., and F. B. Hutt. To appear in this Journal.

Injection of 50 to 100 cc. of egg yolk into the peritoneal cavities of immature female fowls and of capons induced a significant rise in the levels of blood calcium. Similar results were obtained by the use of the estrogenic hormone in the form of progynon-B and as theelin. Removal of 15 to 37 cc. of yolk from laying hens caused a significant drop in the level of blood calcium, the decrease varying from 25 to 48% and being directly proportional in different birds to the amount of yolk removed. Operated controls were unaffected, but in birds in which yolk was squeezed from the follicles and left in the body cavity the serum calcium dropped 23%. The thyrotropic principle of the anterior pituitary depressed blood calcium and an extract of whole anterior pituitary had no effect.

The significance of the operative technic in the removal of the pituitary. Description of a new transphenoidal approach to the pituitary of the dog and notes on the buccal approach to the pituitary in the rabbit. Cope, O., and G. A. Donaldson. To appear in this Journal.

Historical survey of the removal of the pituitary in experimental animals suggests that the early failures to obtain survival were due in part to the operative technic employed. There are still problems of supposed pituitary function wherein considerations of the operative technic are significant. The advantages of a new parapharyngeal approach to the pituitary in the dog are enumerated. The operative technic is given in detail. A description of the modified technic of the parapharyngeal approach in the rabbit is appended.

Experimental phases of the pineal problem. Einhorn, N. H., and L. G. Rowntree. To appear in this Journal.

Pinealectomy in successive generations of parent rats exercises no noteworthy effect in the growth or development of the offspring. The same negative results obtain when either parent alone is pinealectomized. The daily intraperitoneal administration of 1 cc. of pineal extract or of frequent pineal implants in pinealectomized rats exercised no marked effect on the growth and development of the offspring. Frequent pineal implants in normal rats does not affect the rate of growth or development of the offspring. However, the daily intraperitoneal administration of 1 cc. of pineal extract to successive generations of rats has resulted in an increase in the frequency of breeding, an acceleration in the rate of somatic differentiation, and a retardation in the rate of growth in the offspring of treated rats. These biological effects are progressive in character becoming more marked in successive generations under treatment.

A syndrome characterized by premenstrual dermatosis, polyhypermenorrhea, dysmenorrhea, menstrual headache and an increased urinary excretion of sodium pregnanediol glucuronide. Hamblen, E. C. To appear in this Journal.

A patient was described whose symptoms included cyclic ecchymotic lesions during the

latter part of the progestational phase of the cycle, polyhypermenorrhea, dysmenorrhea and menstrual headache. The patient was found to excrete an abnormally large amount of sodium pregnanediol glucuronide in the urine. Intensive estrogenic therapy reduced these abnormal urinary titers and resulted in the disappearance of all the symptoms. An increased formation and metabolism of progesterone was thought to be related to the symptomatology of the patient.

Therapeutic use of the sex sterols in functional meno-metrorrhagia. Hamblen, E. C. To appear in this Journal.

A review of the etiologic factors, ovarian and endometrial correlates and hormonology of functional uterine bleeding has been made. Theories have been advanced that ovarian and endometrial refractivities to gonadotropic and endometriotropic influences, respectively, are etiologic factors in a large group of patients. Therapeutic experiences with gonadotropes of diverse natures and origins have been reviewed. The uniform ineffectiveness of these in altering the pathologic aberrations in the pituitary-ovarian-endometrial system is considered evidence to support the theory of ovarian refractivity. Previously reported observations concerning the effects of the various sex sterols upon active episodes of estrogenic bleeding are summarized: estrogens, and androgens, perhaps, to lesser degree, cause a cessation of estrogenic bleeding; progesterone fails to prevent this bleeding, apparently induces its appearances when given during a non-bleeding phase and often increases the amount of bleeding when given during active flowing. A form of treatment which employs the cyclic administration of estrogen and progesterone or of progesterone alone is described. The observations made upon 12 patients with functional uterine bleeding, who received in all 46 series of these treatments, are reported. During the period of treatments, regular cyclic bleeding occurred and following discontinuation of the injections none of the patients experienced a return of excessive bleeding. Some evidence is presented that these cyclic treatments alter the pathologic aberrations of the pituitary-ovarian-endometrial system, thereby overcoming theoretical ovarian and endometrial refractivity and the associated alterations in the gonadotropic effects of the pituitary.

Sodium pregnanediol glucuronide—the significance of its excretion in the urine. Hamblen, E. C., Catharine Ashley and Margaret Baptist. To appear in this Journal.

The daily urinary excretion of sodium pregnanediol glucuronide was determined by the method of Venning in various women with diverse levels of ovarian function. Certain ones of these were under active endocrine therapy, whereas others were having different surgical procedures. Studies of endometrial specimens obtained by curettage and biopsy were correlated with the hormonal titers. The data secured led to the following conclusions. *A)*, Four factors are concerned in the metabolism of progesterone and in the subsequent urinary excretion of sodium pregnanediol glucuronide: *a)*, ovarian, involving the formation of progesterone by post-ovulational corpora lutea and possibly from the marginal granulosal luteinization of follicles; *b)*, endometrial, concerned with the alteration of progesterone into pregnanediol; *c)*, hepatic, which brings about the conjugation of pregnanediol with glucuronide acid; and *d)* renal, involving the excretion of sodium pregnanediol glucuronide; *B)*, The excretion of this compound indicates the functional capacity of all these factors; the absence of the compound from the urine indicates the functional failure of one or more of the factors; *C)*, A failure of excretion is not conclusive evidence that ovarian function is inadequate; *D)*, The excretion of the pregnanediol-complex is not evidence that an endometrium is undergoing progestational proliferation. *E)*, As a corollary to *D)*, bleeding which occurs from an estrogenic endometrium is not proof that the ovaries are not forming progestin.

A further study of the refractory state produced by adrenal extract. Hartman, F. A., Lena A. Lewis and K. P. McConnell. To appear in this Journal.

A study was made of the conditions necessary for the development of the refractory state from adrenal extracts in dogs, cats and a pig. All animals were kept on a constant regime, including diet. Subcutaneous injections failed to produce the refractory state even after many injections over a long period of time (3 dogs used). There was no indication that the refractory state from intravenous injection was due to haptene formation. An unquestionable refractory state was produced (in one cat and one dog) by intraperitoneal injections. The refractory state is species specific. This was shown by experiments on 4 dogs. The pig (a very young animal) failed to develop the refractory state to either pig or beef adrenal extract. A precaution was suggested for the intravenous injection of adrenal extract in Addison's disease.

Maintenance of the corpus luteum and inhibition of parturition in the rabbit by injection of estrogenic hormone. Heckel G. P., and W. M. Allen. To appear in this Journal.

Parturition in the rabbit is delayed by the continued injection of estrogenic hormone in the latter part of pregnancy. The ovaries must be present for this result to be obtained. In animals in which delivery has been postponed by this means, the corpora lutea are maintained, and their continued activity is thought to be the cause for the delay in parturition. Parturition is not delayed if injections of estrogen are not continued. Isolated injections in the latter part of pregnancy are followed by delivery of dead fetuses within 4 days. Under these circumstances the corpora lutea are not maintained. Cells of such corpora, by actual measurement, are smaller than those from the corpora of normal pregnancy, or from animals in which fetuses have been killed by estrogenic hormone but in which delivery has not occurred because of continuation of the injections. Doses of estrogenic hormone sufficient to delay parturition always cause death of the fetuses. A single injection on the 27th, 28th, or 29th day of pregnancy usually results in death of the fetuses within 27 hours.

Gonadotropic hormone. Clinical application of extraction methods for assay purposes. Heller, C. G., and Emily K. Johnson. To appear in this Journal.

Several concentration methods of urinary gonadotropic hormone for assay purposes were compared with untreated urine to test their completeness of recovery. Several consecutive tests were made from the same pooled specimen to test the regularity of results, and the methods were varied until the simplest satisfactory procedure resulted. The alcohol-ether concentration method essentially fulfills all requirements of an adequate extraction method for clinical use, and has proved its worth in routine assays in this laboratory.

The extent of regeneration of the enucleated adrenal gland in the rat as influenced by the amount of capsule left at operation. Ingle, Dwight J., and George M. Higgins. To appear in this Journal.

In the first experiment the right adrenal gland of fifty pairs of rats was removed from the body. The left adrenal of each rat was enucleated. In one rat of each pair a small amount of adrenal capsule was left intact on the pedicle. In the other rat of each pair a large amount of capsule was left on the pedicle. Pairs of rats were killed at varying periods of time after enucleation of the adrenals and the amounts of regenerated cortical tissue were compared. In the second experiment the right adrenal was removed from fifty rats. The left adrenal was enucleated, varying amounts of the capsule being left intact on the pedicle. After a period of two months the rats were killed. In both experiments a marked relationship was demonstrated between the amount of capsule left at operation and the mass of regenerated cortical tissue.

Fractionation studies on adrenal cortex extract with notes on the distribution of biological activity among the crystalline and amorphous fractions. Kuizenga, M. H., and G. F. Cartland. To appear in this Journal.

Adrenal cortext extract, prepared by the previously reported process of Cartland and Kuizenga, was purified in an ethyl acetate solution by removal of acidic and basic substances, followed by distribution between benzene and water, and finally by fractionation with Girard's reagent T. The distribution of cortical hormone activity was quantitatively followed by the previously described rat method.

Six crystalline substances were isolated, 5 of which are believed to be identical with substances previously reported by Kendall, et al., Wintersteiner and Pfiffner, or Reichstein. The other crystalline substance apparently differs from any previously reported.

Substances E of Kendall and M of Reichstein assayed 1 to 2 R.U. per mg.; A of Kendall (dehydro-cortico-sterone of Reichstein), approximately 3 to 4 R.U. per mg.; the substance, believed to be identical with C of Reichstein, to which biological activity had not previously been ascribed, approximately 3 to 4 R.U. per mg. Substance D of Reichstein and an unidentified substance of melting point 231-234° were found to be practically inactive when assayed at a level of 2 R.U. per mg. The most active of the crystalline substances defined assay, therefore, approximately 3 to 4 R.U. per mg. as compared to 20 to 30 R.U. per mg for certain amorphous fractions obtained.

The inhibitory effect of anti-gonadotropic sera upon endogenous gonadotropic secretion in parabiotic rats. Kupperman, H. S., R. K. Meyer and R. Hertz. To appear in this Journal.

Anti-gonadotropic sera obtained from two adult, female rabbits receiving prolonged injec-

tions of an aqueous, supercentrifuged extract of sheep pituitary, inhibited the effectiveness of the endogenous gonadotropic secretion of the castrate partner's pituitary in 11 female-female and 7 female-male parabiotics when injected into either the castrated or intact partner. The inhibitory sera was of sufficient titer to prevent the ovarian hypertrophy and the extensive luteinization found in the control series.

Induction of mating in the dog with pregnancy urine extract. Leathem, J. H., and J. A. Morrell. To appear in this Journal.

Pregnancy urine extract was found to be capable of inducing some external manifestations of estrus in the normal dog, namely, vulval swelling and mating, but proestrus bleeding was generally absent. Induction of mating was successful with mature dogs in anestrum in all of the 11 cases tested, whereas the castrate failed to respond. Follicular stimulation to small and medium-sized follicles resulted, and after injections were discontinued corpora lutea atretica formed. Some endometrial hypertrophy also resulted. The changes produced failed to duplicate the normal cycle, because, *a)*, the vaginal smears did not correspond; *b)*, the endometrium developed only to an early estrus state and then regressed; *c)*, a pseudopregnant period did not follow mating as shown by the failure of a pseudopregnant endometrium to develop. Furthermore, only a small amount of lutein tissue formed in most cases, to be maintained for only a short period, and finally, *d)*, ovulation did not occur.

The biological assay of the mammogenic duct growth factor of the anterior pituitary. Lewis, A. A., C. W. Turner and E. T. Gomez. To appear in this Journal.

A study is reported on the use of male albino mice in the biological assay of the mammary duct growth factor of prehypophyses (mammogen). Large doses of gonadotropic hormone were entirely negative for mammary growth stimulation, as was male sex hormone for at least the first 16 days of injection. The injections of oreton were rather heavy, and should surpass the male hormone which could be developed under stimulation of injections of fresh pituitary tissue. This question is to be further investigated. The influence of varying daily dosages of estrogen and prehypophyseal tissue from pregnant cattle, and of length of injection period was studied. It was concluded that the convenient period of 6 days was satisfactory. The proposed mouse assay unit for the mammogenic duct growth factor is the total amount of tissue or extract required to produce definite signs of development in one or more glands of 50-10% of 10 male albino mice weighing 10 to 25 gm. Injection should be made once daily, subcutaneously, for 6 successive days, sacrificing on the seventh. The reproducibility of the assay method was shown by results obtained with 6 groups of mice. Four groups were within the proposed limit, whereas one was definitely outside the limit. Twenty mice in a group would increase the accuracy of the results.

The release of colloid (thyroglobulin) from the thyroid gland by centrifugal force. McClendon, J. F. To appear in this Journal.

The colloid of the thyroid follicle appears to be a sol of stored thyroglobulin. It was released by a centrifugal force of 100,000 to 200,000 times gravity. The cells of the follicle are heavier than the colloid and are thrown through it but are held by the connective tissue and basement membrane of the follicular epithelium on the centrifugal side. Since thyroglobulin has a very high molecular weight (nearly 700,000) it moves centrifugally, but displacement by the cells causes the sol to move centripetally and evidently passes between the cells, although no pores may be seen in fixed tissues. In other words, as the follicular epithelium is collapsed by centrifugal force the colloid is released from the lumen of the follicle, forcing its way between the cells.

Atypical uterine growths produced by the prolonged administration of oestrogenic hormones. Nelson, Warren O. To appear in this Journal.

The effects of the prolonged administration of oestrogenic hormone upon the female guinea pig has been studied. One hundred and thirty-seven animals injected for periods ranging from three weeks to fourteen months are considered in this report. Daily dosages ranged from 350 to 700 I.U. A regular finding in short-time experiments has been thhe occurrence of cystic glandular hyperplasia. More prolonged treatment has resulted in the appearance of marked adenomatous hyperplasia and metaplasia. The latter was most striking in the cervical regions, but showed a tendency to be present throughout the fundi and cornua. Frequently, although not invariably, fibromyomatous growths of varying size were found on the uteri. These are predominantly fibroid in character. A significant, although not entirely explained phenomenon,

has been the frequent occurrence of irregular periods of vaginal bleeding. These periods appear to be associated with fluctuations in the level of estrogenic hormone.

Protamine insulin: three practical points. Robson, G. B., and H. Gray. To appear in this Journal.

Forty patients treated with protamine insulin were analyzed. The diet averaged C 135 gm., P 72 gm., F 84 gm., Cal. 1500. Lumps, or subcutaneous nodules about 1 cm. diameter followed the injection in 20 patients, i.e., 50%; these were attributable in about half the instances not to the brand, or to allergy, but to the short (½ inch) needle. The others remained unexplained. Reactions occurred in 25 patients, i.e., 62%. This high frequency was attributed to, *a)*, efforts to keep the urine sugar-free, unlike authors who let patients run some sugar, and, *b)*, close questioning in order to miss none. Large single dosages of protamine insulin at breakfast seems unduly to be feared; one patient took 90 U, another 120 U repeatedy without mishap.

The relationship of hemopoietic phenomena to endocrine disorders in women. Sharp, E. A., and H. C. Mack. To appear in this Journal.

The universal interest in disorders of the blood and in endocrinopathies has developed an imposing bibliography on both subjects. There is a paucity of expression, however, as to whether there is a reciprocal relationship between endocrine disease and hemopoietic disturbance, particularly anemia. The data in this report propose that the coexistence of an anemia and endocrine disorders in women constitutes a clinical syndrome, and further, re-emphasize Osler's dictum that secondary anemia is a sequel to, rather than an antecedent of, disease.

The diabetes of bearded women. Suprarenal tumor, diabetes, and hirsutism. Shepardson, Clare, and W. Shapiro. To appear in this Journal.

Recent investigation into the rôle of the suprarenal cortex in the metabolism of carbohydrates attaches more than historical value to a reconsideration of the diabetes of bearded women (suprarenal tumor, diabetes, and hirsutism). The 17 unequivocal examples of this syndrome in the literature are summarized, and the 18th case is added. The possible relationship of the suprarenal cortical tumor to the diabetes is discussed in the light of recent experimental data. It is concluded that in this syndrome the suprarenal function is but a single, although probably morbific, element of a multiple glandular disturbance involving, in addition the hormonogenic portion of the pancreas, the ovaries, and possibly the adenohypophysis.

Chick thyroid response as a basis for thyrotropic hormone assay. Smelser, G. K. To appear in this Journal.

Day-old white leghorn chicks are found to be exceptionally well suited for use in assays of thyrotropic hormone. An increase in thyroid weight may be produced by one-tenth the amount of hormone required to affect the weight of guinea-pig thyroids. Chick thyroids respond, by growth, to a wide range of dosages. A maximum response is reached with 100 times the minimal stimulating dose whereas, in the guinea pig, a maximum response is obtained with 8 times the minimum. Chick thyroids are capable of a weight increase of 10-11 times; the guinea-pig thyroids only 4-5 times their original weight in a 5-day period. The weight response of chick thyroids to thyrotropic extracts is consistent, and significant values can be obtained with small groups. Marked histological changes accompany the weight increase of the treated chick thyroids, and should be studied if weight increase is slight. Chicks tolerate but do not react (by thyroid-weight increase) to injections of crude tissue extracts, blood serum or urine. Therefore, chicks may be used to test the thyrotropic activity of these substances. They are inexpensive and easy to house and feed. Among other advantages is the fact that an unlimited supply of the same age, weight and standard stock may easily be obtained.

A new and improved method for the preparation of pineal extract. Steinberg, Arthur. To appear in this Journal.

A method is described for the preparation of biologically active pineal extract. The material is a dilute hydrochloric acid extract of acetone defatted pineal glands of beef. The final product is protein free and thermostable, and does not alter appreciably on ageing. It contains no pressor or depressor substances, and may be employed in large dosages with impunity.

Progestin studies 1—pregnanediol excretion. Stover, R. F., and J. P. Pratt. To appear in this Journal.

Quantitative determinations of pregnanediol excreted in the urine of 14 normal and 7

abnormal women were made according to the method of Venning and Browne. Pregnanediol was found in measurable quantities during the lutein phase of normal menstrual cycle as well as in dysmenorrhea. The amount excreted increases throughout pregnancy and disappears rapidly following the interruption of pregnancy. A wide variation in the amount of pregnanediol excreted in different individuals and in different cycles of the same individual limit the diagnostic value of a single determination. No striking change from the normal was found in cases of dysmenorrhea of threatened abortion.

Thyroid function in diabetes insipidus in the rat. Swann, H. G., and P. E. Johnson. To appear in this Journal.

In a study of the participation of the thyroid in the diabetes insipidus following removal of the posterior hypophysis in the rat and its exacerbation by saline solutions, it has been found that: (1) the basal metabolic rate and the size of the splanchnic viscera are normal in such rats; hence, presumably, the thyroid is not over-active; (2) a posthypophysectomy following thyroidectomy results in only a light diabetes insipidus, but (3) a thyroidectomy following saline-exacerbated operative diabetes affects only slightly and transiently the fluid exchange; (4) a thyroid enlargement occurs in extreme saline diabetes insipidus, but histologically the gland does not appear hyperactive; (5) thyroid administration, as others have found, does not influence the rat's fluid exchange, but (6) if thyroid administration is combined with saline drinking solutions, it causes in the rat an increased and persistent fluid exchange.

The effect of salts on the diabetes insipidus following posthypophysectomy in the rat. Swann, H. G., and B. J. Penner. To appear in this Journal.

NaCl, given in the drinking solutions, causes extreme aggravation of the diabetes insipidus following removal of the posterior hypophysis in rats. In concentrated solutions, it may even cause the animals' deaths. It also prevents the spontaneous recovery from the operative diabetes insipidus seen in the rat. Pituitrin prevents these saline aggravations. Mammalian Ringer's solution and $NaHCO_3$ are similarly diuretic, but Na_2SO_4, Na citrate, KCl and $CaCl_2$ exert no diuretic effects on the diabetic rats beyond their effects on the normal rat. A working hypothesis is presented that NaCl plays an important rôle in the diabetes insipidus following posthypophysectomy.

Lactogenic hormone:—severe reactions from its use in the human. Werner, A. A. To appear in this Journal.

The author treated 5 castrate girls with theelin and theelin and progesterone to stimulate development of the ducts and the acini of the breasts. As much as 84,000 I.U. of theelin-in-oil was administered intramuscularly over a period of 21 days. During the last 7 days of this period 10 RB.U. of progesterone was added daily to the injections. Theelin effect on the breasts was characterized by development of the gland trees, enlargement, a sensation of fullness, tingling and erection of the nipples. Progesterone exaggerated the theelin effect. Lactogenic hormone injections, which had to be discontinued before completion of the experiment because of severe local and anaphylactic reactions, caused the breasts to become fuller and more tense, with a sensation of trickling in the ducts and tingling in the nipples. It is concluded that lactogenic hormone preparations offered for human use, at present, are unsafe. Further research to find the cause of untoward reactions and to purify the lactogenic extracts is indicated.

ADRENALS

The rôle of epinephrine in the lack of response to insulin in diphtheria intoxication. Beamer, C., and G. S. Eadie, Am. J. Physiol. 122:627. 1938.

Healthy rabbits weighing 2-3 kg. were used in the experiments. Insulin in doses of 0.37-0.51 U. per kg. was given in the control curves. 1.25-1.5 M.L.D. of diphtheria toxin per kg. was given subcutaneously. On the morning of the 3rd day insulin in the same dosage was given, and the blood-sugar curve again followed. In most cases insulin failed to lower the blood sugar of the animals thus poisoned. When ergotoxin was given, this power was restored. When epinephrin was given intravenously in doses of 0.1 cc. of 1:1000 solution, a somewhat diminished effect on the blood sugar of the toxin-treated animals was noted. The authors concluded that the toxin markedly lowered the threshold for stimuli which induce the secretion of epinephrin.—Lena Lewis.

Status lymphaticus: Diagnosis and treatment preliminary to surgical procedures. Campbell, E. H., Pennsylvania M. J. 41:907. 1938.

The author finds that correlation of published material on status lymphaticus establishes it as a definite clinical entity. The clinical picture and experimental and post-mortem evidence point to deficiency of the adrenal hormones as the etiologic factor. The condition appears to be closely associated with allergy. Diagnosis is difficult and is dependent on the usual signs and symptoms, with emphasis on the general appearance of the patient, the enlarged thymus, allergic signs, and characteristics of adrenal insufficiency. The treatment of suspected cases is chiefly by use of extracts of the adrenal gland substance, although some aid may be obtained also by proper regulation of the diet and climate, enforced rest, and use of certain drugs. Patients requiring surgical procedures should undergo the indicated prophylactic measures against accidents.—I. B.

Calcium and potassium in the blood after excision and grafting of adrenals (Studio sul comportamento del calcio e del potasio nel siero di sangue dopo asportazione e dopo inesto di surrene). Donato, D., Sperimentale, Arch. di biol. 92:267. 1938.

A total of 22 rabbits was used in these experiments. Unilateral adrenalectomy caused an initial drop in the blood calcium from 14 mg. per 100 cc. to 13-9.9 mg. within 4 to 10 days, followed by an increase up to 16 mg. Implantation of fresh adrenal gland into the abdominal cavity produced an immediate increase of Ca by 2-3 mg. Bilateral adrenalectomy, performed in 2 animals, led to death within 8 hours preceded by a drop in blood Ca by 5.5 and 8 mg. A two-stage operation was tolerated with a variable drop in Ca after each operation. Adrenalin injection increased the calcium by approximately 3 mg. for about a week, which was followed by a decrease. The effect of the potassium was exactly reciprocal to the calcium. The initial values, as reported, varied between 8.94 and 33.96 mg. and the changes varied accordingly within wide limits.—A. E. M.

Survival of adrenalectomized toads (La supervivencia de los sapos suprarrenalectomizados). Fustinoni, O., Rev. Soc. argent. biol. 14:40. 1938.

The adrenals were destroyed by diathermo-coagulation in toads *(Bufo arenarum)*. The maximum survival observed in summer was 5 days and 14 days in winter; no difference was observed between male and female animals. Maintained in a damp atmosphere at 35°C., all animals died in the course of the first day; at 5°C. they survived up to 14 days. Damp atmospheres were more favorable than dry ones. Survival was not prolonged by implantation of fresh adrenal glands from toads nor by injection of NaCl (1-5-10%); glucose (10-20%) sodium bicarbonate, vitamin B_1, adrenalin, extract of anterior hypophysis, adrenal gland extract and cortin.—J. T. L.

Treatment of marasmus by injection of extract of adrenal cortex. Hislop, W. A., Lancet 235:308. 1938.

The author tested out the hypothesis that whatever the original cause of marasmus may be, there was ultimately an adrenal hypofunction, and until this is corrected recovery may be retarded or impossible. The use of adrenal extract therefore would tend to tide over the period of adrenal insufficiency and permit the utilization of adequate diet. Accordingly, two series of infants furnished the basis of experiments; one group (5 unselected cases) was given adrenal cortex, the results suggested adrenal hypofunction to the author. A second group (14 infants) definitely diagnosed as having marasmus was put on encortone (Allen & Hanbury, Ltd.) given daily in intramuscular doses of 1 minim per 2 lb. of body weight. The procedure followed was a two-week control period, encortone given for 14 days, then a break of 10-14 days in treatment, followed by an intensive treatment period of 6-10 weeks. This form of therapy resulted in a gain of weight and a fall in marasmus death rate.—L. B. S.

Metabolism and body temperature of normal and adrenalectomized rats during exposure to cold. Ring, G. C., Am. J. Physiol. 122:435. 1938.

The metabolism, as determined by the Benedict closed circuit apparatus and the body temperature as determined by a thermocouple placed in the colon was approximately the same at both 37° and 5-7°C. for partially adrenalectomized rats as for normals. The critical thermal increment of normal rats for O_2 consumption is 16,100. Adrenalectomized rats have the same value for rising body temperature but a higher value for falling body temperatures. It is concluded that the adrenal cortex has nothing to do with the 'master' reaction for oxidation at the temperatures studied.—F. A. Hitchcock.

The estimation of adrenaline. Shaw, F. H., Biochem. J. 32:19. 1938.

Adrenalin reduces arsenomolybdic acid to a blue compound which is measured colorimetrically; by this means adrenalin can be estimated in tissues which contain it in amounts exceeding 10 γ per 100 gm.—D. L. T.

Synthetic desoxycorticosterone acetate in Addison's disease. Simpson, S. L., Lancet 235:557. 1938.

Two patients with Addison's disease were injected intramuscularly with desoxycorticosterone acetate in oil solution (Organon Laboratories). The one with severe adrenal deficiency gave an equivocal response, the other was benefited to a greater degree than when receiving cortin. One cc. of desoxycorticosterone acetate was found to be equivalent to more than 5 cc. and less than 20 cc. of cortin.—L. B. S.

Chemical structure of corticosterone. Steiger, Marguerite, and T. Reichstein, Nature. 141: 202. 1938.

Corticosterone has been converted into the hydrocarbon allo-pregnane (obtainable also from allo-pregnandial) and is thus proved to possess a steroid structure.—D. L. T.

Action of nicotine upon the blood pressure in rabbits, deprived of the coeliac and superior mesenteric ganglia and the suprarenals. Tiba, M., Tohoku J. Exper. Med. 33: 213. 1938.

Experiments were made on rabbits to determine the action of nicotine upon their blood pressure after suprarenalectomy and the removal of their celiac and superior mesenteric ganglia. Nicotine in doses of 0.5 mg. and 1 mg. per kilo of body weight was given intravenously to rabbits thus operated upon and to normal ones. Neither the fall of the blood pressure occurring immediately after the injectin of nicotine nor its subsequent elevation was changed by the surgical procedures.—Margaret Baptist.

Presence of an antagonistic factor in serum of dogs following repeated injections of cortin. Toby, C. Gwendoline, and Lena A. Lewis, Proc. Soc. Exper. Biol. & Med. 37:352. 1937.

Repeated injections of cortin into each of 5 dogs led to progressively decreasing responses in electrolyte excretion until the dogs became completely refractory to the injected material. The potent extracts, which were protein-free, gave precipitin reactions with sera from the refractory animals but not with sera from normal dogs. There was some indication also of complement fixation in the reaction of refractory sera with the antigen.—M. H. F.

A further study of the augmenting action of nicotine upon the epinephrin secretion from the suprarenals of the non-anaesthetized dog. Wada, M., H. Takuji and M. Tiba, Tohoku J. Exper. Med. 33:189. 1938.

Experiments were made on dogs to ascertain the influence of nicotine upon the output of epinephrin, the blood pressure and the blood sugar. Dogs whose D_{10}-L_2 dorsal spinal roots had been severed bilaterally were used. The blood from the suprarenal vein was collected before the injection of nicotine, during the first and the second 30 seconds after the start of the injection and at intervals thereafter. The concentration of epinephrin was estimated on the rabbit by the intestinal method. The blood pressure was taken from the femoral artery laid bare under local anesthesia with 0.5% novocain solution. Nicotine (Merck) was injected into the saphenous vein in a dose of 1 mg. per kilo of body weight in a dilution of 1:500. It was found that the greatest output of epinephrin occurred 30 seconds after starting the injection of nicotine; after this point it diminished rapidly. The blood pressure fell a the time of the greatest output of epinephrin, after which it began to rise again. The rise and subsequent fall in the blood sugar occurred more slowly than that in the blood pressure.—Margaret Baptist.

ENDOCRINE-GENERAL

The effect of parenteral injection of purines, methylated purines, and various drugs upon creatine-creatinine metabolism. Beard, H. H., and P. Pizzolato, J. Pharmacol. & Exper. Therap. 63:306. 1938.

In rats, pituitrin did not increase the muscle creatine above normal. Adrenalin and thyroxine, in dosages of 0.1 and 1.0 mg. per 100 gm. of body weight, caused a decrease in muscle creatine of 13 and 47% respectively.—E. L.

Rôle of advanced paternal age in causing Mongolism. A Study of 2822 cases. Bleyer, A., Amer. J. Dis. Child. 55:79. 1938.

Upon the basis of this study the author concludes that the rôle of advanced maternal

age as a causative factor is undeniable. Satisfactory evidence could not be found of etiological relationship of the following: advanced paternal age; immaturity of either parent; primogeniture, ultimogeniture; birth rank; the broad differences in the ages of the parents; the prolonged interval preceding the birth of the mongoloid child; low fertility and reproductive exhaustion of each parent. It is probable that the apparent relation of any of these factors to mongolism can be explained through admittance of advanced maternal age.—M. B. G.

Laurence-Moon-Beidl syndrome. Cooperstock, M., Am. J. Pediat. **54**:334. 1937.

The general clinical features of the Laurence-Moon-Beidl syndrome are briefly discussed, and a family is described in which 5 of 13 children of one generation were affected. Two of these 5 children came under observation; in the family history the remaining 3 gave strong evidence of the syndrome, except that genital hypoplasia was questionable. In the second patient, retinitis pigmentosa, a constant finding in this condition, was absent, but in a child of 2 years its ultimate appearance is still possible.—M. B. G.

Endocrine obesity in children: clinical and laboratory studies and results of treatment. Gordon, M. B., J. Pediat. **10**:204. 1937.

The author believes that obesity in children is most frequently a result not of overeating per se, but of endocrine disturbances, most frequently hypothyroidism and hypopituitarism. A classification of these disturbances and a table for differential diagnosis between thyroid and pituitary types of obesity are given. Fifty children (29 boys and 21 girls) with endocrine obesity were studied and observed for at least 6 months to 1 year. The etiologic factors represented in the series are, *a)*, hypopituitarism in 86% (primary in 48%, with secondary hypothyroidism in 38%); *b)*, hypothyroidism in 12% (primary in 8%, with secondary hypopituitarism in 4%); c*)*, hypogonadism (primary female) in 2%. Sexual development was retarded in 27 boys. Cryptorchidism was present in 5 unilaterally and in 7 bilaterally. Menstrual disturbances were present in 9 girls of post-pubertal age, including one of primary eunuchoidism. On the basis of actual weight, a B.M.R. below —10 was noted in 55%, between +10 and —10 in 41%, and above +10 in 4%. The specific dynamic action was found to be diminished. Blood chemistry studies indicated that with endocrine obesity chlorides and uric acid are high, cholesterol high normal (depending upon the degree of accompanying hypothyroidism, sugar low and urea nitrogen and creatinine normal. Salt and water retention were present in the majority of cases. The treatment in this series included prescription of a diet low in calories; restriction of salt and water intake; administration of desiccated thyroid, anterior pituitary gland substance, and extract of the urine of pregnant women or of an estrogen in cryptorchidism, menstrual disorders and protracted hypogonadism. The results of treatment were reduction in weight to normal or to within 10% overweight for height and age on the basis of accrued height; redistribution of fat to a more normal allocation; a general increase in height; an average increase in the B.M.R. of from 8 to 5; an increase in specific dynamic action, and improvement in hypogonadism and cryptorchidism, which were influenced to a greater extent than menstrual disorders.—Author's Abst.

Girdle type adiposity among mentally deficient males. Hecker, A. O., and V. C. Warren, Am. J. Dis. Child. **54**:1257. 1938.

Twelve cases of girdle type adiposity occurred in 1608 mentally deficient boys in a school for mental defectives. Evidence is offered to support the hypothesis that the Laurence-Moon-Beidl syndrome and the primary type of the Fröhlich syndrome are the result of defective germ plasm, that the Laurence-Moon-Beidl syndrome is not a distinct clinical entity but a symptom complex varying in its manifestations with the degree of defect in the parental germ plasm and that the syndromes studied differ in the degree rather than in the kind of defect. It is suggested that in the Laurence-Moon-Beidl syndrome the manifestations are secondary to the mental defect and are the result of the defect in the germ plasm which produces the mental defect. Cases heretofore classified as cases of Fröhlich's syndrome with no regard for etiologic factors should be divided into primary and secondary cases, depending on the presence or absence of demonstrable pathologic change as the cause of the signs and symptoms.—M. B. G.

The endocrine factors in human sterility. An evaluation of diagnostic and therapeutic measures. Mazer, C., S. L. Israel and C. W. Charny, Pennsylvania M.J. **41**:1009. 1938.

Involuntary sterility is on the increase in all civilized countries. This, coupled with the current tendency to small families, becomes a social and medical problem of mounting importance. The incidence of involuntary barrenness in this country is placed at 13% by E. Rey-

nolds and D. Macomber, and at 17% by A. Lotka. A condition so prevalent and so destructive to the happiness of marital life certainly deserves serious attention. No marital union should be considered infertile before the lapse of 2 years of non-use of contraceptives or the occurrence of 2 consecutive spontaneous abortions. Of the 389 adequately studied barren couples, 104 women and 35 men were found to have major defects of endocrine origin. In most of them, other factors, unrelated to the endocrine system, contributed largely to the unproductivness of the marital unions. Even in the absence of lower genital tract infection, massage of the seminal vesicles and prostate twice weekly for a period of 6 months is the most effective single measure in the treatment of male infertility. Reflex stimulation of the spermatogenic apparatus probably accounts for the clinical results obtained with this procedure. Low-dosage irradiation of the pituitary gland and ovaries, within certain limits, is the most effective single agent in the treatment of amenorrhea or dysfunctional uterine bleeding as the major etiologic factor in sterility. Endocrine therapy simultaneously employed increases materially the percentage of cures. In the non-endocrine group of sterile women, repeated transuterine insufflation and office dilatation of the cervix give the best results.—I. B.

Osteodystrophy fibrosa. Report of a case in which the condition was combined with precocious puberty, pathologic pigmentation of the skin and hyperthyroidism, with a review of the literature. McCune, D. J., and H. Bruch, Am. J. Dis. Child. 54:806. 1937.

The authors report a case in a 9-year-old girl who presented pubertas praecox, asymmetrically distributed fibrocystic osteodystrophy and pigmentation of the skin. These features had been apparent since the end of the second year of life, and in addition evidence of hyperthyroidism was present since 4 years of age.

Numerous chemical examinations of the blood gave normal results except for increase of plasma phosphatase. Calcium balance studies gave normal results. Parathyroid origin was ruled out because of lack of definite information obtained by biopsy of bone and skin and parathyroid hormone determination. Gonadotropic and estrogen determination also gave negative results.

The authors analyzed 9 other similar cases in female children in the literature and in 5 males. The latter did not manifest any pubertas praecox or advanced ossification. They considered that a common third factor is responsible for the syndrome.—M. B. G.

Endocrine therapy in acne vulgaris. Templeton, H. J., and S. R. Truman, California and West. Med. 48:337. 1938.

This is a study of a series of patients, all college students, treated with estrogenic substance. The authors conclude that, although a certain number of these patients showed improvement, the results were certainly far inferior to orthodox dermatologic-roentgen therapy. And yet the improvement which did result encourages the authors in their belief that acne is related to the endocrine system, and that further experiments with endocrine therapy are in order. However, when only 50% or less show any improvement and none shows complete remission, the physician who undertakes the treatment of an individual with APL substance of PU or estrogenic substances is promising less chance of cure than the average patient is willing to take. Until it is possible to determine which gland preparation to use, which patient will respond, and how much to give, it would seem wise to refer endocrine therapy in acne back to the field of research problems.—I. B.

On the antianemic function of the small intestine. Uotila, U., Acta med. Scandinav. 95:415. 1938.

Sections of the small intestine taken from the duodenum, the jejunum, and the lower part of the ileum, were ground, defatted with benzol, dried and pulverized. Twenty gm. amounts of the various preparations were fed to 10 patients with pernicious anemia twice daily. It was found that the small intestine contained an active antianemic substance throughout the length. The intestine seemed to contain more active material than the stomach, and the greatest effect appeared with ileum preparations.—J. M. L.

GONADS

Parallel determination of urinary oestrogens in the pregnant mare by the Cuboni reaction and the Allen-Doisy test (Vergleichende Bestimmung der Starke der Ostrogenen wirkung des Harns von Trachtigen Stuten mit der Cuboni-Reaktion und dem Allen-Doisy-Test). Anersen, I., and K. Pedersen-Bjergaard, Klin. Wchnschr. 17:91. 1938.

The Cuboni reaction (green fluorescence with conc. sulphuric acid) was positive in

urines containing 40,000 to 50,000 U of estrogenic activity after hydrolysis. The reaction was negative or uncertain in lesser concentrations. Estrone gave a positive color reaction at a concentration of 8 γ per cc. Estriol did not react in this concentration. The intensity of the color reaction, however, was no guide to the concentration of estrogenic activity of the urine.—M. H. F.

Clinical experiment in estrin therapy. Bishop, P. M. F., Brit. M. J. 1:939. 1938. Abst., J.A.M.A. 111:208

Estrogen is now well established as a therapeutic agent, but many problems remain to be solved before the most effective mode of administration can be determined. Bishop describes the effect of estrogen on a castrated woman (ovariectomy for cysts). Administration of estrogen in quite small doses by mouth was effective in controlling symptoms of hot flashes. The level of theelin threshold bleeding was found to be between 6,000 and 5,000 I.U. by injection and between 25,000 and 30,000 I.U. by mouth, suggesting a peroral: intramuscular ratio of 5:1. A 14 mg. tablet of crystalline theelin was implanted subcutaneously and was effective in controlling symptoms for 4 or 5 weeks.

Synthetic male hormone re prostatic hypertrophy. Cary, F. S., Illinois M.J. 73:486. 1938.

The author concludes that in a large percentage of cases testosterone has a definite place in the treatment of early benign hypertrophy of the prostate. In inoperable cases, due to serious physical disability, testosterone should be given a fair trial in early prostatism. Five mg. doses are recommended.—I. B.

Menstruation, orally induced. Evidences of the Activity of Progynon-C, a new follicle hormone derivative, and Proluton-C, a new luteal hormone preparation, after oral administration (Menstruation-per os erzeugt. Beweise de Wirksamkeit von Progynon C, einem neuen Follikelhormonderivat, und Proluton C, einem neuen Luteohormonpräparat, bei peroraler Verabreichung). Clauberg, C., and Z. Uestün, Zentrlbl. f. Gynäk. 62:1745. 1938.

Using the new synthetic derivatives of estrin and progestin prepared by Hohlweg and Inhoffen, called progynon-C and proluton-C, the authors demonstrate by x-rays with contrast media that infantile uteri can be made to grow noticeably in the course of one month. Curette specimens showed proliferative changes in endometrium, and flows followed, in cases with amenorrhea. There were evidences of tubal growth. The intensive treatment with proluton-C gave results showing progestational development, with improvement in cases having cystic hyperplasia of the endometrium. Doses employed were 2 to 3 times those which Clauberg had used by intramuscular injection, with estrin 6 times those employed with progestin.—E. L. S.

The experimental production of ovulation in the human subject. Davis, M. E., and A. K. Koff, Am. J. Obst. & Gynec. 36:183. 1938.

By intravenous injection of 50 to 90 U of pregnant mare serum gonadotropic material ovulation was produced in the various stages of the menstrual cycle. In 36 cases the ovaries were examined grossly and microscopically. Usually the endometrium was found still in the proliferative phase. Some cases showed more than one ovulation following the treatment. The corpora lutea showed evidence of recent intensive stimulation by gonadotropic substance. Ovulation was brought about in 3 women with metrorrhagia.—E. L. S.

Factors influencing the effectiveness of administered hormones. Deanesly, Ruth, and A. S. Parkes, Proc. Roy. Soc. London, s.B., 124:279. 1937.

Various androgenic and estrogenic hormones and their esters were tested, using the weight of the accessory glands of castrated male rats, the area of the capon's comb, the capon's plumage, and the uterine weight of immature rabbits as test objects. The effect of subdivision of the dose, of the nature of the solvent, the addition of fatty substances to the medium and the volume thereof, the route of administration, etc., were studied. All these variables influence the results, and the extent and nature of the influence varies unpredictably from one substance tested to another: a method ideal for one substance may be inefficient for another of similar physiological action. Some of the free hormones may effectively be given in the form of tablets or crystals implanted subcutaneously; the solid masses may be recovered subsequently and reweighed. The efficiency of many esters seems to depend on slow absorption from the site of injection and vanishes when intraperitoneal or intravenous administration is used; the free hormones may be as efficient as the esters, when absorption is as slow as it is from a solid pellet.—D. L. T.

Estimation of (capon) comb growth hormone in urine. Dingemanse, Elisabeth, and E. Laqueur, Biochem. J. **32**:651. 1938.

Combined androgens in urine may be hydrolyzed by heating with 150 cc. 25% HCl per litter, in the presence of benzene, without destroying any activity.—D. L. T.

Chemical identification of oestrone in human male urine. Dingemanse, Elisabeth, E. Laqueur and O. Mühlbock, Nature **141**:927. 1938.

Estrone has been isolated in pure form from human male urine, in which it makes up one-third to one-half of the total estrogenic activity.—D. L. T.

The effect of sex hormones on lactation in the rat. Folley, S. J., and S. K. Kon, Proc. Roy. Soc. London, s.B., **124**:476. 1938.

Lactation is inhibited, as shown by decreased growth of the litters, by administration of estradiol monobenzoate (0.3 mg. per 100 gm. per day), or testosterone propionate (0.4 mg.), but not of progesterone or androsterone (0.3 mg.). Rats ovariectomized after parturition continue to lactate, and show decreased sensitiveness to estradiol.—D. L. T.

Ovotrophic and folliculophasic hormones. Freud, J., Nature **141**:1013. 1938.

The action of anterior pituitary hormones on cartilage, mammary gland, adrenal cortex, corpus luteum, thyroid, Leydig cells, etc., is in general plasmotrophic: that is, it causes tissue growth by cellular hypertrophy rather than by stimulating mitosis and proliferation. The stimulation of Graafian follicles is an apparent exception to this rule: but it is shown, firstly that mitoses continue around medium-sized ova for weeks after hypophysectomy in the rat, and secondly that the frequency of mitosis in follicles (determined by 'trapping' nuclei in mitosis with colchicine) is correlated with the size and degree of development of the contained ovum, in rat, mouse, guinea-pig, rabbit and dog. It is concluded that the pituitary FSH is in reality ovum-stimulating, the ovum itself then producing a 'folliculophasic' hormone which provokes proliferation in the surrounding granulosa cells.—D. L. T.

The effect of estrogenic hormone upon the contractility of the fallopian tubes. Geist, S. H., U. J. Salmon and M. Mintz, Am. J. Obst. & Gynec. **36**:67. 1938.

After the menopause there is gradual but marked impairment of the tone of the fallopian tube musculature and disappearance of rhythmic peristalsis. The demonstration is by reduced resistance to the entrance of gas, kymographically recorded. The changes vary in proportion to the time since the menopause. Vaginal smears showed deficiency of estrin in proportion to tubal inactivity. Administration of estrin caused the appearance of rhythmic contractions of high amplitude similar to those seen in normal women, with coincident vaginal estrin responses. In the normal human estrin is held responsible for the rhythmic tubal contractions. These relations may be of significance in some forms of sterility.—E. L. S.

Endocrine study of hypogonadism and cryptorchidism. Gordon, M. B., New York State J. Med. **36**:1313. 1936.

In a review of the records of 521 boys with endocrine or non-endocrine conditions, the author found that hypogonadism and cryptorchidism occur almost twice as frequently in the endocrine as in the nonendocrine group. Studies on 38 boys with cryptorchidism and 36 with hypogonadism with descended testes show on the average a thyropituitary deficiency as manifested by low B.M.R., low specific dynamic action of proteins and high blood values of cholesterol, chlorides and uric acid. The treatment of cryptorchidism in this series is based on the assumption that it is a symptom in the majority of instances of a disturbed thyropituitary-gonad relationship and not a clinical entity. Three methods are used: *a)*, oral administration of thyroid and APE in increasing dosage; *b)*, oral administration of thyroid and APE and hypodermic injection of APE and, *c)*, oral administration of thyroid and APE and hpyodermic injection of APL extract. Complete descent of the testes was obtained with the 3 methods as follows: first, 33%; second, 50%; third, 57%. The treatment of cryptorchidism by any of the methods used may be effectual at any age between 2 and 14 years but is more effective after 5 years. No boy should be subjected to an operation for undescended testicles until he has been given the benefit of organotherapy for at least 6 months. Hypogonadism, especially when associated with adiposogenital dystrophy and hypothyroidism, responds in the majority of instances to oral administration of thyroid and anterior pituitary extracts and hypodermic injections of APE. Extract of PU is necessary only in the stubborn and protracted cases. Improvement in the B.M.R., specific dynamic action, general endocrine condition and obesity is generally but not always accompanied by the same degree of improvement in the hypogonadism and cryptorchidism. The effects on the phallus, scrotum and testes are not parallel. Untoward effects may result from the use of either anterior pituitary or APL extracts.